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PROVISIONAL SPECIFICATION

Applicant:

BIONOMICS LIMITED

A.C.N. 075 582 740

Invention Title:

NUCLEIC ACID MOLECULES ASSOCIATED WITH ANGIOGENESIS II

The invention is described in the following statement:

NUCLEIC ACID MOLECULES ASSOCIATED WITH ANGIOGENESIS II

Technical Field

The present invention relates to novel nucleic acid sequences ("angiogenic genes") involved in the process of angiogenesis. Each of the angiogenic genes encode a polypeptide that has a role in angiogenesis. In view of the realisation that these genes play a role in angiogenesis, the invention is also concerned with the therapy of pathologies associated with angiogenesis, the screening of drugs for pro- or anti-angiogenic activity, the diagnosis and prognosis of pathologies associated with angiogenesis, and in some cases the use of the nucleic acid sequences to identify and obtain full-length angiogenesis-related genes.

Background Art

The formation of new blood vessels from pre-existing vessels, a process termed angiogenesis, is essential for normal growth. Important angiogenic processes include those taking place in embryogenesis, renewal of the endometrium, formation and growth of the corpus luteum of pregnancy, wound healing and in the restoration of tissue structure and function after injury.

The formation of new capillaries requires a co-ordinated series of events mediated through the expression of multiple genes which may have either pro- or anti-angiogenic activities. The process begins with an angiogenic stimulus to existing vasculature, usually mediated by growth factors such as vascular endothelial growth factor or basic fibroblast growth factor. This is followed by degradation of the extracellular matrix, cell adhesion changes (and disruption), an increase in cell permeability, proliferation of endothelial cells (ECs) and migration of ECs towards the site of blood vessel formation. Subsequent processes include capillary tube or

lumen formation, stabilisation and differentiation by the migrating ECs.

In the (normal) healthy adult, angiogenesis is virtually arrested and occurs only when needed. However, a number of pathological situations are characterised by enhanced, uncontrolled angiogenesis. These conditions include cancer, rheumatoid arthritis, diabetic retinopathy, psoriasis and cardiovascular diseases such as atherosclerosis. In other pathologies such as ischaemic limb disease or in coronary artery disease, growing new vessels through the promotion of an expanding vasculature would be of benefit.

A number of *in vitro* assays have been established which are thought to mimic angiogenesis and these have provided important tools to examine the mechanisms by which the angiogenic process takes place and the genes most likely to be involved.

Lumen formation is a key step in angiogenesis. The presence of vacuoles within ECs undergoing angiogenesis have been reported and their involvement in lumen formation has been postulated (Folkman and Haudenschild, 1980; Gamble et al., 1993). The general mechanism of lumen formation suggested by Folkman and Haudenschild (1980), has been that vacuoles form within the cytoplasm of a number of aligned ECs which are later converted to a tube. The union of adjacent tubes results in the formation of a continuous unicellular capillary lumen. However, little is known about the changes in cell morphology leading to lumen formation or the signals required for ECs to construct this feature.

An *in vitro* model of angiogenesis has been created from human umbilical vein ECs plated onto a 3 dimensional collagen matrix (Gamble et al., 1993). In the presence of phorbol myristate acetate (PMA) these cells form capillary tubes within 24 hours. With the addition of anti-integrin antibodies, the usually unicellular tubes (thought to reflect an immature, poorly differentiated phenotype) are

converted to form a multicellular lumen through the inhibition of cell-matrix interactions and promotion of cell-cell interactions. This model has subsequently allowed the investigation of the morphological events which occur in lumen formation.

For the treatment of diseases associated with angiogenesis, understanding the molecular genetic mechanisms of the process is of paramount importance. The use of the *in vitro* model described above (Gamble et al., 1993), a model that reflects the critical events that occur during angiogenesis *in vivo* in a time dependant and broadly synchronous manner, has provided a tool for the identification of the key genes involved.

A number of genes have been identified from this model to be differentially expressed during the angiogenesis process. Functional analysis of a subset of these angiogenic genes and their effect on endothelial cell function and proliferation is described in detail below.

The isolation of these angiogenic genes has provided novel targets for the treatment of angiogenesis-related disorders.

Disclosure of the Invention

The present invention provides isolated nucleic acid molecules, which have been shown to be regulated in their expression during angiogenesis (see Table 1).

In a first aspect of the present invention there is provided isolated nucleic acid molecules as defined by Figures 1 to 44.

Following the realisation that the molecules listed in Table 1 are regulated in their expression during angiogenesis, the invention provides isolated nucleic acid molecules as laid out in Table 1, or fragments thereof, that play a role in an angiogenic process. Such a process may include, but is not restricted to, embryogenesis, menstrual cycle, wound repair, tumour angiogenesis and

exercise induced muscle hypertrophy.

In addition, the present invention provides isolated nucleic acid molecules as laid out in Table 1 (hereinafter referred to as "angiogenic genes", "angiogenic nucleic acid molecules" or "angiogenic polypeptides" for the sake of convenience), or fragments thereof, that play a role in diseases associated with the angiogenic process. Diseases may include, but are not restricted to, cancer, rheumatoid arthritis, diabetic retinopathy, psoriasis, cardiovascular diseases such as atherosclerosis, ischaemic limb disease and coronary artery disease.

The invention also encompasses an isolated nucleic acid molecule that is at least 70% identical to any one of the angiogenic genes of the invention and which plays a role in the angiogenic process.

Such variants will have preferably at least about 85%, and most preferably at least about 95% sequence identity to the angiogenic genes. Any one of the polynucleotide variants described above can encode an amino acid sequence, which contains at least one functional or structural characteristic of the relevant angiogenic gene of the invention.

Sequence identity is typically calculated using the BLAST algorithm, described in Altschul *et al* (1997) with the BLOSUM62 default matrix.

The invention also encompasses an isolated nucleic acid molecule which hybridises under stringent conditions with any one of the angiogenic genes of the invention and which plays a role in an angiogenic process.

Hybridisation with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, may be used to identify nucleic acid sequences which encode the relevant angiogenic gene. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridisation or amplification will

determine whether the probe identifies only naturally occurring sequences encoding the angiogenic gene, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably have at least 50% sequence identity to any of the angiogenic gene encoding sequences of the invention. The hybridisation probes of the subject invention may be DNA or RNA and may be derived from any one of the angiogenic gene sequences or from genomic sequences including promoters, enhancers, and introns of the angiogenic genes.

Means for producing specific hybridisation probes for DNAs encoding any one of the angiogenic genes include the cloning of polynucleotide sequences encoding the relevant angiogenic gene or its derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, and are commercially available. Hybridisation probes may be labelled by radionuclides such as ^{32}P or ^{35}S, or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, or other methods known in the art.

Under stringent conditions, hybridisation with ^{32}P labelled probes will most preferably occur at 42°C in 750 mM NaCl, 75 mM trisodium citrate, 2% SDS, 50% formamide, 1X Denhart's, 10% (w/v) dextran sulphate and 100 µg/ml denatured salmon sperm DNA. Useful variations on these conditions will be readily apparent to those skilled in the art. The washing steps which follow hybridisation most preferably occur at 65°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art.

The nucleotide sequences of the present invention can be engineered using methods accepted in the art so as to alter angiogenic gene-encoding sequences for a variety of purposes. These include, but are not limited to, modification of the cloning, processing, and/or expression

of the gene product. PCR reassembly of gene fragments and the use of synthetic oligonucleotides allow the engineering of angiogenic gene nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis can introduce mutations that create new restriction sites, alter glycosylation patterns and produce splice variants etc.

As a result of the degeneracy of the genetic code, a number of polynucleotide sequences encoding the angiogenic genes of the invention, some that may have minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention includes each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of the naturally occurring angiogenic gene, and all such variations are to be considered as being specifically disclosed.

The polynucleotides of this invention include RNA, cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified, as will be appreciated by those skilled in the art. Such modifications include labels, methylation, intercalators, alkylators and modified linkages. In some instances it may be advantageous to produce nucleotide sequences encoding an angiogenic gene or its derivatives possessing a substantially different codon usage than that of the naturally occurring gene. For example, codons may be selected to increase the rate of expression of the peptide in a particular prokaryotic or eukaryotic host corresponding with the frequency that particular codons are utilized by the host. Other reasons to alter the nucleotide sequence encoding an angiogenic gene or its derivatives without altering the encoded amino acid sequence include the production of RNA transcripts

having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of the nucleic acid molecules of the invention, entirely by synthetic chemistry. Synthetic sequences may be inserted into expression vectors and cell systems that contain the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements may include regulatory sequences, promoters, 5' and 3' untranslated regions and specific initiation signals (such as an ATG initiation codon and Kozak consensus sequence) which allow more efficient translation of sequences encoding the angiogenic genes. In cases where the complete coding sequence including its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, additional control signals may not be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals as described above should be provided by the vector. Such signals may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used (Scharf et al., 1994).

Nucleic acid molecules that are complements of the sequences described herein may also be prepared.

The present invention allows for the preparation of purified polypeptides or proteins. In order to do this, host cells may be transfected with a nucleic acid molecule as described above. Typically, said host cells are transfected with an expression vector comprising a nucleic acid molecule according to the invention. A variety of expression vector/host systems may be utilized to contain and express the sequences. These include, but are not limited to, microorganisms such as bacteria transformed with plasmid or cosmid DNA expression vectors; yeast

transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); or mouse or other animal or human tissue cell systems. Mammalian cells can also be used to express a protein that is encoded by a specific angiogenic gene of the invention using various expression vectors including plasmid, cosmid and viral systems such as a vaccinia virus expression system. The invention is not limited by the host cell employed.

The polynucleotide sequences, or variants thereof, of the present invention can be stably expressed in cell lines to allow long term production of recombinant proteins in mammalian systems. Sequences encoding any one of the angiogenic genes of the invention can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. The selectable marker confers resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode a protein may be designed to contain signal sequences which direct secretion of the protein through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, glycosylation, phosphorylation, and acylation. Post-translational cleavage of a "prepro" form of the protein may also be

used to specify protein targeting, folding, and/or activity. Different host cells having specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO or HeLa cells), are available from the American Type Culture Collection (ATCC) and may be chosen to ensure the correct modification and processing of the foreign protein.

When large quantities of protein are needed such as for antibody production, vectors which direct high levels of expression may be used such as those containing the T5 or T7 inducible bacteriophage promoter. The present invention also includes the use of the expression systems described above in generating and isolating fusion proteins which contain important functional domains of the protein. These fusion proteins are used for binding, structural and functional studies as well as for the generation of appropriate antibodies.

In order to express and purify the protein as a fusion protein, the appropriate polynucleotide sequences of the present invention are inserted into a vector which contains a nucleotide sequence encoding another peptide (for example, glutathionine succinyl transferase). The fusion protein is expressed and recovered from prokaryotic or eukaryotic cells. The fusion protein can then be purified by affinity chromatography based upon the fusion vector sequence and the relevant protein can subsequently be obtained by enzymatic cleavage of the fusion protein.

Fragments of polypeptides of the present invention may also be produced by direct peptide synthesis using solid-phase techniques. Automated synthesis may be achieved by using the ABI 431A Peptide Synthesizer (Perkin-Elmer). Various fragments of polypeptide may be synthesized separately and then combined to produce the full length molecule.

In instances where the isolated nucleic acid molecules of the invention represent only partial gene sequence, these partial sequences can be used to obtain

the corresponding sequence of the full-length angiogenic gene. Therefore, the present invention further provides the use of a partial nucleic acid molecule of the invention comprising a nucleotide sequence defined by any one of the sequences defined in figure 1 to 44 to identify and/or obtain full-length human genes involved in the angiogenic process. Full-length angiogenic genes may be cloned using the partial nucleotide sequences of the invention by methods known *per se* to those skilled in the art. For example, *in silico* analysis of sequence databases such as those hosted at the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) can be searched in order to obtain overlapping nucleotide sequence. This provides a "walking" strategy towards obtaining the full-length gene sequence. Appropriate databases to search at this site include the expressed sequence tag (EST) database (database of GenBank, EMBL and DDBJ sequences from their EST divisions) or the non redundant (nr) database (contains all GenBank, EMBL, DDBJ and PDB sequences but does not include EST, STS, GSS, or phase 0, 1 or 2 HTGS sequences). Typically searches are performed using the BLAST algorithm described in Altschul *et al* (1997) with the BLOSUM62 default matrix. In instances where *in silico* "walking" approaches fail to retrieve the complete gene sequence, additional strategies may be employed. These include the use of "restriction-site PCR" which allows the retrieval of unknown sequence adjacent to a portion of DNA whose sequence is known. In this technique universal primers are used to retrieve unknown sequence. Inverse PCR may also be used, in which primers based on the known sequence are designed to amplify adjacent unknown sequences. These upstream sequences may include promoters and regulatory elements. In addition, various other PCR-based techniques may be used, for example a kit available from Clontech (Palo Alto, California) allows for a walking PCR technique, the 5'RACE kit (Gibco-BRL) allows isolation of additional 5'

gene sequence, while additional 3' sequence can be obtained using practised techniques (for eg see Gecz et al., 1997).

The present invention also provides isolated polypeptides, which have been shown to be regulated in their expression during angiogenesis (see Table 1).

More specifically, following the realisation that these polypeptides are regulated in their expression during angiogenesis, the invention provides isolated polypeptides as laid out in Table 1, or fragments thereof, that play a role in an angiogenic process. Such a process may include, but is not restricted to, embryogenesis, menstrual cycle, wound repair, tumour angiogenesis and exercise induced muscle hypertrophy.

In addition, the present invention provides isolated polypeptides as laid out in Table 1, or fragments thereof, that play a role in diseases associated with the angiogenic process. Diseases may include, but are not restricted to, cancer, rheumatoid arthritis, diabetic retinopathy, psoriasis, cardiovascular diseases such as atherosclerosis, ischaemic limb disease and coronary artery disease.

The invention also encompasses an isolated polypeptide having at least 70%, preferably 85%, and more preferably 95%, identity to any one of the polypeptides as laid out in Table 1, and which plays a role in an angiogenic process.

Sequence identity is typically calculated using the BLAST algorithm, described in Altschul *et al* (1997) with the BLOSUM62 default matrix.

In a further aspect of the invention there is provided a method of preparing a polypeptide as described above, comprising the steps of:

(1) culturing the host cells under conditions effective for production of the polypeptide; and

(2) harvesting the polypeptide.

According to still another aspect of the invention

there is provided a polypeptide which is the product of the process described above.

Substantially purified protein or fragments thereof can then be used in further biochemical analyses to establish secondary and tertiary structure for example by x-ray crystallography of the protein or by nuclear magnetic resonance (NMR). Determination of structure allows for the rational design of pharmaceuticals to interact with the protein, alter protein charge configuration or charge interaction with other proteins, or to alter its function in the cell.

The invention has provided a number of genes likely to be involved in angiogenesis. As angiogenesis is critical in a number of pathological processes, the invention therefore enables therapeutic methods for the treatment of all angiogenesis-related disorders, and may enable the diagnosis or prognosis of all angiogenesis-related disorders associated with abnormalities in expression and/or function of any one of the angiogenic genes.

Examples of such disorders include, but are not limited to, cancer, rheumatoid arthritis, diabetic retinopathy, psoriasis, cardiovascular diseases such as atherosclerosis, ischaemic limb disease and coronary artery disease.

According to another aspect of the present invention there is provided a method of treating an angiogenesis-related disorder as described above, comprising administering a selective agonist or antagonist of an angiogenic gene or protein of the invention to a subject in need of such treatment.

Still further there is provided the use of a selective agonist or antagonist of an angiogenic gene or protein of the invention for the treatment of an angiogenesis-related disorder as described above.

For the treatment of angiogenesis-related disorders which result in uncontrolled or enhanced angiogenesis,

including but not limited to, cancer, rheumatoid arthritis, diabetic retinopathy, psoriasis and cardiovascular diseases such as atherosclerosis, therapies which inhibit the expanding vasculature are desirable. This would involve inhibition of any one of the angiogenic genes or proteins that are able to promote angiogenesis, or enhancement, stimulation or re-activation of any one of the angiogenic genes or proteins that are able to inhibit angiogenesis.

For the treatment of angiogenesis-related disorders which are characterised by inhibited or decreased angiogenesis, including but not limited to, ischaemic limb disease and coronary artery disease, therapies which enhance or promote vascular expansion are desirable. This would involve inhibition of any one of the angiogenic genes or proteins that are able to restrict angiogenesis or enhancement, stimulation or re-activation of any one of the angiogenic genes or proteins that are able to promote angiogenesis.

Inhibiting gene or protein function

Inhibiting the function of a gene or protein can be achieved in a variety of ways. Antisense nucleic acid methodologies represent one approach to inactivate genes whose altered expression is causative of a disorder. In one aspect of the invention an isolated nucleic acid molecule, which is the complement of any one of the relevant angiogenic nucleic acid molecules described above and which encodes an RNA molecule that hybridises with the mRNA encoded by the relevant angiogenic gene of the invention, may be administered to a subject in need of such treatment. Typically, a complement to any relevant one of the angiogenic genes is administered to a subject to treat or prevent an angiogenesis-related disorder.

In a further aspect of the invention there is provided the use of an isolated nucleic acid molecule which is the complement of any one of the relevant nucleic

acid molecules of the invention and which encodes an RNA molecule that hybridises with the mRNA encoded by the relevant angiogenic gene of the invention, in the manufacture of a medicament for the treatment of an angiogenesis-related disorder.

Typically, a vector expressing the complement of a polynucleotide encoding any one of the relevant angiogenic genes may be administered to a subject to treat or prevent an angiogenesis-related disorder including, but not limited to, those described above. Many methods for introducing vectors into cells or tissues are available and equally suitable for use *in vivo*, *in vitro*, and *ex vivo*. For *ex vivo* therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art (for example, see Goldman *et al.*, 1997).

Additional antisense or gene-targeted silencing strategies may include, but are not limited to, the use of antisense oligonucleotides, injection of antisense RNA, transfection of antisense RNA expression vectors, and the use of RNA interference (RNAi) or short interfering RNAs (siRNA). Still further, catalytic nucleic acid molecules such as DNAzymes and ribozymes may be used for gene silencing (Breaker and Joyce, 1994; Haseloff and Gerlach, 1988). These molecules function by cleaving their target mRNA molecule rather than merely binding to it as in traditional antisense approaches.

In a further aspect purified protein according to the invention may be used to produce antibodies which specifically bind any relevant angiogenic protein of the invention. These antibodies may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues that express the relevant angiogenic protein. Such

antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric and single chain antibodies as would be understood by the person skilled in the art.

For the production of antibodies, various hosts including rabbits, rats, goats, mice, humans, and others may be immunized by injection with a protein of the invention or with any fragment or oligopeptide thereof, which has immunogenic properties. Various adjuvants may be used to increase immunological response and include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface-active substances such as lysolecithin. Adjuvants used in humans include BCG (bacilli Calmette-Guerin) and Corynebacterium parvum.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to the relevant angiogenic protein have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of amino acids from these proteins may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to any relevant angiogenic protein may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (For example, see Kohler et al., 1975; Kozbor et al., 1985; Cote et al., 1983; Cole et al., 1984).

Monoclonal antibodies produced may include, but are not limited to, mouse-derived antibodies, humanised antibodies and fully human antibodies.

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (For example, see Orlandi et al., 1989; Winter et al., 1991).

Antibody fragments which contain specific binding sites for any relevant angiogenic protein may also be generated. For example, such fragments include, F(ab')2 fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (For example, see Huse et al., 1989).

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between a protein and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes is preferred, but a competitive binding assay may also be employed.

In a further aspect, antagonists may include peptides, phosphopeptides or small organic or inorganic compounds. These antagonists should disrupt the function of any relevant angiogenic gene of the invention so as to provide the necessary therapeutic effect.

Peptides, phosphopeptides or small organic or inorganic compounds suitable for therapeutic applications may be identified using nucleic acids and polypeptides of the invention in drug screening applications as described below.

Enhancing gene or protein function

Enhancing, stimulating or re-activating a gene's or protein's function can be achieved in a variety of ways. In one aspect of the invention administration of an isolated nucleic acid molecule, as described above, to a subject in need of such treatment may be initiated. Typically, any relevant angiogenic gene of the invention can be administered to a subject to treat or prevent an angiogenesis-related disorder.

In a further aspect, there is provided the use of an isolated nucleic acid molecule, as described above, in the manufacture of a medicament for the treatment of an angiogenesis-related disorder.

Typically, a vector capable of expressing any relevant angiogenic gene, or a fragment or derivative thereof, may be administered to a subject to treat or prevent a disorder including, but not limited to, those described above. Transducing retroviral vectors are often used for somatic cell gene therapy because of their high efficiency of infection and stable integration and expression. Any relevant full-length gene, or portions thereof, can be cloned into a retroviral vector and expression may be driven from its endogenous promoter or from the retroviral long terminal repeat or from a promoter specific for the target cell type of interest. Other viral vectors can be used and include, as is known in the art, adenoviruses, adeno-associated viruses, vaccinia viruses, papovaviruses, lentiviruses and retroviruses of avian, murine and human origin.

Gene therapy would be carried out according to established methods (Friedman, 1991; Culver, 1996). A vector containing a copy of any relevant angiogenic gene linked to expression control elements and capable of replicating inside the cells is prepared. Alternatively the vector may be replication deficient and may require helper cells for replication and use in gene therapy.

Gene transfer using non-viral methods of infection *in*

vitro can also be used. These methods include direct injection of DNA, uptake of naked DNA in the presence of calcium phosphate, electroporation, protoplast fusion or liposome delivery. Gene transfer can also be achieved by delivery as a part of a human artificial chromosome or receptor-mediated gene transfer. This involves linking the DNA to a targeting molecule that will bind to specific cell-surface receptors to induce endocytosis and transfer of the DNA into mammalian cells. One such technique uses poly-L-lysine to link asialoglycoprotein to DNA. An adenovirus is also added to the complex to disrupt the lysosomes and thus allow the DNA to avoid degradation and move to the nucleus. Infusion of these particles intravenously has resulted in gene transfer into hepatocytes.

Although not identified to date, it is possible that certain individuals with angiogenesis-related disorders contain an abnormality in any one of the angiogenic genes of the invention. Therefore, in affected subjects that have decreased expression or activity of an angiogenic gene, a mechanism of down-regulation may be due to abnormal methylation of promoter regions of those angiogenic genes which contain CpG islands. Therefore in an alternative approach to therapy, administration of agents that remove abnormal promoter methylation may reactivate gene expression and restore normal function to the affected cell.

In affected subjects that express a mutated form of any one of the angiogenic genes of the invention it may be possible to prevent the disorder by introducing into the affected cells a wild-type copy of the gene such that it recombines with the mutant gene. This requires a double recombination event for the correction of the gene mutation. Vectors for the introduction of genes in these ways are known in the art, and any suitable vector may be used. Alternatively, introducing another copy of the gene bearing a second mutation in that gene may be employed so

as to negate the original gene mutation and block any negative effect.

In a still further aspect, there is provided a method of treating an angiogenesis-related disorder comprising administering a polypeptide, as described above, or an agonist thereof, to a subject in need of such treatment.

In another aspect the invention provides the use of a polypeptide as described above, or an agonist thereof, in the manufacture of a medicament for the treatment of an angiogenesis-related disorder. Examples of such disorders are described above.

In a further aspect, a suitable agonist may also include peptides, phosphopeptides or small organic or inorganic compounds that can mimic the function of any relevant angiogenic gene, or may include an antibody to any relevant angiogenic gene that is able to restore function to a normal level.

Peptides, phosphopeptides or small organic or inorganic compounds suitable for therapeutic applications may be identified using nucleic acids and polypeptides of the invention in drug screening applications as described below.

In further embodiments, any of the agonists, antagonists, complementary sequences, nucleic acid molecules, proteins, antibodies, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents may be made by those skilled in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, therapeutic efficacy with lower dosages of each agent may be possible, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including,

for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Drug screening

According to still another aspect of the invention, nucleic acid molecules of the invention as well as peptides of the invention, particularly any relevant purified angiogenic polypeptides or fragments thereof, and cells expressing these are useful for screening of candidate pharmaceutical compounds in a variety of techniques for the treatment of angiogenesis-related disorders.

Still further, it provides the use wherein high throughput screening techniques are employed.

Compounds that can be screened in accordance with the invention include, but are not limited to peptides (such as soluble peptides), phosphopeptides and small organic or inorganic molecules (such as natural product or synthetic chemical libraries and peptidomimetics).

In one embodiment, a screening assay may include a cell-based assay utilising eukaryotic or prokaryotic host cells that are stably transformed with recombinant nucleic acid molecules expressing the relevant angiogenic polypeptide or fragment, in competitive binding assays. Binding assays will measure for the formation of complexes between the relevant polypeptide or fragments thereof and the compound being tested, or will measure the degree to which a compound being tested will interfere with the formation of a complex between the relevant polypeptide or fragment thereof, and its interactor or ligand.

Non cell-based assays may also be used for identifying compounds that interrupt binding between the polypeptides of the invention and their interactors. Such assays are known in the art and include for example AlphaScreen technology (PerkinElmer Life Sciences, MA, USA). This application relies on the use of beads such that each interaction partner is bound to a separate bead

via an antibody. Interaction of each partner will bring the beads into proximity, such that laser excitation initiates a number of chemical reactions ultimately leading to fluorophores emitting a light signal. Candidate compounds that disrupt the binding of the relevant angiogenic polypeptide with its interactor will result in no light emission enabling identification and isolation of the responsible compound.

High-throughput drug screening techniques may also employ methods as described in WO84/03564. Small peptide test compounds synthesised on a solid substrate can be assayed through relevant angiogenic polypeptide binding and washing. The relevant bound angiogenic polypeptide is then detected by methods well known in the art. In a variation of this technique, purified angiogenic polypeptides can be coated directly onto plates to identify interacting test compounds.

An additional method for drug screening involves the use of host eukaryotic cell lines which carry mutations in any relevant angiogenic gene of the invention. The host cell lines are also defective at the polypeptide level. Other cell lines may be used where the gene expression of the relevant angiogenic gene can be regulated (i.e. over-expressed, under-expressed, or switched off). The host cell lines or cells are grown in the presence of various drug compounds and the rate of growth of the host cells is measured to determine if the compound is capable of regulating the growth of defective cells.

The angiogenic polypeptides of the present invention may also be used for screening compounds developed as a result of combinatorial library technology. This provides a way to test a large number of different substances for their ability to modulate activity of a polypeptide. The use of peptide libraries is preferred (see WO 97/02048) with such libraries and their use known in the art.

A substance identified as a modulator of polypeptide function may be peptide or non-peptide in nature. Non-

peptide "small molecules" are often preferred for many *in vivo* pharmaceutical applications. In addition, a mimic or mimetic of the substance may be designed for pharmaceutical use. The design of mimetics based on a known pharmaceutically active compound ("lead" compound) is a common approach to the development of novel pharmaceuticals. This is often desirable where the original active compound is difficult or expensive to synthesise or where it provides an unsuitable method of administration. In the design of a mimetic, particular parts of the original active compound that are important in determining the target property are identified. These parts or residues constituting the active region of the compound are known as its pharmacophore. Once found, the pharmacophore structure is modelled according to its physical properties using data from a range of sources including x-ray diffraction data and NMR. A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be added. The selection can be made such that the mimetic is easy to synthesise, is likely to be pharmacologically acceptable, does not degrade *in vivo* and retains the biological activity of the lead compound. Further optimisation or modification can be carried out to select one or more final mimetics useful for *in vivo* or clinical testing.

It is also possible to isolate a target-specific antibody and then solve its crystal structure. In principle, this approach yields a pharmacophore upon which subsequent drug design can be based as described above. It may be possible to avoid protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analogue of the original binding site. The anti-id could then be used to isolate peptides from chemically or biologically produced peptide banks.

Another alternative method for drug screening relies on structure-based rational drug design. Determination of the three dimensional structure of the polypeptides of the invention, or the three dimensional structure of the protein complexes which may incorporate these polypeptides allows for structure-based drug design to identify biologically active lead compounds.

Three dimensional structural models can be generated by a number of applications, some of which include experimental models such as x-ray crystallography and NMR and/or from *in silico* studies using information from structural databases such as the Protein Databank (PDB). In addition, three dimensional structural models can be determined using a number of known protein structure prediction techniques based on the primary sequences of the polypeptides (e.g. SYBYL - Tripos Associated, St. Louis, MO), *de novo* protein structure design programs (e.g. MODELER - MSI Inc., San Diego, CA, or MOE - Chemical Computing Group, Montreal, Canada) or *ab initio* methods (e.g. see US Patent Numbers 5331573 and 5579250).

Once the three dimensional structure of a polypeptide or polypeptide complex has been determined, structure-based drug discovery techniques can be employed to design biologically-active compounds based on these three dimensional structures. Such techniques are known in the art and include examples such as DOCK (University of California, San Francisco) or AUTODOCK (Scripps Research Institute, La Jolla, California). A computational docking protocol will identify the active site or sites that are deemed important for protein activity based on a predicted protein model. Molecular databases, such as the Available Chemicals Directory (ACD) are then screened for molecules that complement the protein model.

Using methods such as these, potential clinical drug candidates can be identified and computationally ranked in order to reduce the time and expense associated with typical 'wet lab' drug screening methodologies.

Compounds identified from the screening methods described above form a part of the present invention, as do pharmaceutical compositions containing these and a pharmaceutically acceptable carrier.

Pharmaceutical Preparations

Compounds identified from screening assays as indicated above can be administered to a patient at a therapeutically effective dose to treat or ameliorate a disorder associated with angiogenesis. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of the disorder.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The data obtained from these studies can then be used in the formulation of a range of dosages for use in humans.

Pharmaceutical compositions for use in accordance with the present invention can be formulated in a conventional manner using one or more physiological acceptable carriers, excipients or stabilisers which are well known. Acceptable carriers, excipients or stabilizers are non-toxic at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including absorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; binding agents including hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or non-ionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).

The formulation of pharmaceutical compositions for use in accordance with the present invention will be based on the proposed route of administration. Routes of administration may include, but are not limited to, inhalation, insufflation (either through the mouth or nose), oral, buccal, rectal or parental administration.

Diagnostic and prognostic applications

Should abnormalities in any one of the angiogenic genes of the invention exist, which alter activity and/or expression of the gene to give rise to angiogenesis-related disorders, the polynucleotides and polypeptides of the invention may be used for the diagnosis or prognosis of these disorders, or a predisposition to such disorders. Examples of such disorders include, but are not limited to, cancer, rheumatoid arthritis, diabetic retinopathy, psoriasis, cardiovascular diseases such as atherosclerosis, ischaemic limb disease and coronary artery disease. Diagnosis or prognosis may be used to determine the severity, type or stage of the disease state in order to initiate an appropriate therapeutic intervention.

In another embodiment of the invention, the polynucleotides that may be used for diagnostic or prognostic purposes include oligonucleotide sequences, genomic DNA and complementary RNA and DNA molecules. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which abnormal expression or mutations in any one of the angiogenic genes may be correlated with disease. Genomic DNA used for the diagnosis or prognosis may be obtained from body cells, such as those present in the blood, tissue biopsy, surgical specimen, or autopsy material. The DNA may be isolated and used directly for detection of a specific sequence or may be amplified by the polymerase chain reaction (PCR) prior to analysis. Similarly, RNA or cDNA may also be used, with or without PCR amplification. To

detect a specific nucleic acid sequence, direct nucleotide sequencing, reverse transcriptase PCR (RT-PCR), hybridisation using specific oligonucleotides, restriction enzyme digest and mapping, PCR mapping, RNAse protection, and various other methods may be employed. Oligonucleotides specific to particular sequences can be chemically synthesized and labelled radioactively or nonradioactively and hybridised to individual samples immobilized on membranes or other solid-supports or in solution. The presence, absence or excess expression of any one of the angiogenic genes may then be visualized using methods such as autoradiography, fluorometry, or colorimetry.

In a particular aspect, the nucleotide sequences of the invention may be useful in assays that detect the presence of associated disorders, particularly those mentioned previously. The nucleotide sequences may be labelled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridisation complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis or prognosis of an angiogenesis-related disorder associated with a mutation in any one of the angiogenic genes of the invention, the nucleotide sequence of the relevant gene can be compared between normal tissue and diseased tissue in order to establish whether the patient expresses a mutant gene.

In order to provide a basis for the diagnosis of a disorder associated with abnormal expression of any one of the angiogenic genes of the invention, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding the relevant angiogenic gene, under conditions suitable for hybridisation or amplification. Standard hybridisation may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Another method to identify a normal or standard profile for expression of any one of the angiogenic genes is through quantitative RT-PCR studies. RNA isolated from body cells of a normal individual, particularly RNA isolated from endothelial cells, is reverse transcribed and real-time PCR using oligonucleotides specific for the relevant gene is conducted to establish a normal level of expression of the gene. Standard values obtained in both these examples may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridisation assays or quantitative RT-PCR studies may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

According to a further aspect of the invention there is provided the use of an angiogenic polypeptide as described above in the diagnosis or prognosis of an angiogenesis-related disorder associated with any one of angiogenic genes of the invention, or a predisposition to

such disorders.

When a diagnostic or prognostic assay is to be based upon any relevant angiogenic polypeptide, a variety of approaches are possible. For example, diagnosis or prognosis can be achieved by monitoring differences in the electrophoretic mobility of normal and mutant proteins. Such an approach will be particularly useful in identifying mutants in which charge substitutions are present, or in which insertions, deletions or substitutions have resulted in a significant change in the electrophoretic migration of the resultant protein. Alternatively, diagnosis or prognosis may be based upon differences in the proteolytic cleavage patterns of normal and mutant proteins, differences in molar ratios of the various amino acid residues, or by functional assays demonstrating altered function of the gene products.

In another aspect, antibodies that specifically bind the relevant angiogenic gene product may be used for the diagnosis or prognosis of disorders characterized by abnormal expression of the gene, or in assays to monitor patients being treated with the relevant angiogenic gene or protein or agonists, antagonists, or inhibitors thereof. Antibodies useful for diagnostic or prognostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic or prognostic assays may include methods that utilize the antibody and a label to detect the relevant protein in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labelled by covalent or non-covalent attachment of a reporter molecule.

A variety of protocols for measuring the relevant angiogenic polypeptide, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of expression. Normal or standard values for expression are established by combining body fluids or cell extracts taken from normal

mammalian subjects, preferably human, with antibody to the relevant protein under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, preferably by photometric means. Quantities of protein expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

Once an individual has been diagnosed or prognosed with a disorder, effective treatments can be initiated, as described above. In the treatment of angiogenesis-related diseases which are characterised by uncontrolled or enhanced angiogenesis, the expanding vasculature needs to be inhibited. This would involve inhibiting the relevant angiogenic genes or proteins of the invention that promote angiogenesis. In addition, treatment may also need to stimulate expression or function of the relevant angiogenic genes or proteins of the invention whose normal role is to inhibit angiogenesis but whose activity is reduced or absent in the affected individual.

In the treatment of angiogenesis-related diseases which are characterised by inhibited or decreased angiogenesis, approaches which enhance or promote vascular expansion are desirable. This may be achieved using methods essentially as described above but will involve stimulating the expression or function of the relevant angiogenic gene or protein whose normal role is to promote angiogenesis but whose activity is reduced or absent in the affected individual. Alternatively, inhibiting genes or proteins that restrict angiogenesis may also be an approach to treatment.

Microarray

In further embodiments, complete cDNAs, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used

as probes in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose or prognose a disorder, and to develop and monitor the activities of therapeutic agents. Microarrays may be prepared, used, and analysed using methods known in the art. (For example, see Schena et al., 1996; Heller et al., 1997).

Transformed hosts

The present invention also provides for the production of genetically modified (knock-out, knock-in and transgenic), non-human animal models transformed with the nucleic acid molecules of the invention. These animals are useful for the study of the function of the relevant angiogenic gene, to study the mechanisms of disease as related to these genes, for the screening of candidate pharmaceutical compounds, for the creation of explanted mammalian cell cultures which express the protein or mutant protein and for the evaluation of potential therapeutic interventions.

Animal species which are suitable for use in the animal models of the present invention include, but are not limited to, rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs, and non-human primates such as monkeys and chimpanzees. For initial studies, genetically modified mice and rats are highly desirable due to the relative ease in generating knock-in, knock-out or transgenics of these animals, their ease of maintenance and their shorter life spans. For certain studies, transgenic yeast or invertebrates may be suitable and preferred because they allow for rapid screening and provide for much easier handling. For longer term studies, non-human primates may be desired due to their similarity with humans.

To create an animal model based on any one of the angiogenic genes of the invention, several methods can be employed. These include generation of a specific mutation in a homologous animal gene, insertion of a wild type human gene and/or a humanized animal gene by homologous recombination, insertion of a mutant (single or multiple) human gene as genomic or minigene cDNA constructs using wild type or mutant or artificial promoter elements, or insertion of artificially modified fragments of the endogenous gene by homologous recombination. The modifications include insertion of mutant stop codons, the deletion of DNA sequences, or the inclusion of recombination elements (lox p sites) recognized by enzymes such as Cre recombinase.

To create transgenic mice in order to study gain of gene function *in vivo*, any relevant angiogenic gene can be inserted into a mouse germ line using standard techniques such as oocyte microinjection. Gain of gene function can mean the overexpression of a gene and its protein product, or the genetic complementation of a mutation of the gene under investigation. For oocyte injection, one or more copies of the wild type or mutant gene can be inserted into the pronucleus of a just-fertilized mouse oocyte. This oocyte is then reimplanted into a pseudo-pregnant foster mother. The liveborn mice can then be screened for integrants using analysis of tail DNA for the presence of the relevant human angiogenic gene sequence. The transgene can be either a complete genomic sequence injected as a YAC, BAC, PAC or other chromosome DNA fragment, a cDNA with either the natural promoter or a heterologous promoter, or a minigene containing all of the coding region and other elements found to be necessary for optimum expression.

To generate knock-out mice or knock-in mice, gene targeting through homologous recombination in mouse embryonic stem (ES) cells may be applied. Knock-out mice are generated to study loss of gene function *in vivo* while

knock-in mice allow the study of gain of function or to study the effect of specific gene mutations. Knock-in mice are similar to transgenic mice however the integration site and copy number are defined in the former.

For knock-out mouse generation, gene targeting vectors can be designed such that they delete (knock-out) the protein coding sequence of the relevant angiogenic gene in the mouse genome. In contrast, knock-in mice can be produced whereby a gene targeting vector containing the relevant angiogenic gene can integrate into a defined genetic locus in the mouse genome. For both applications, homologous recombination is catalysed by specific DNA repair enzymes that recognise homologous DNA sequences and exchange them via double crossover.

Gene targeting vectors are usually introduced into ES cells using electroporation. ES cell integrants are then isolated via an antibiotic resistance gene present on the targeting vector and are subsequently genotyped to identify those ES cell clones in which the gene under investigation has integrated into the locus of interest. The appropriate ES cells are then transmitted through the germline to produce a novel mouse strain.

In instances where gene ablation results in early embryonic lethality, conditional gene targeting may be employed. This allows genes to be deleted in a temporally and spatially controlled fashion. As above, appropriate ES cells are transmitted through the germline to produce a novel mouse strain, however the actual deletion of the gene is performed in the adult mouse in a tissue specific or time controlled manner. Conditional gene targeting is most commonly achieved by use of the cre/lox system. The enzyme cre is able to recognise the 34 base pair loxP sequence such that loxP flanked (or floxed) DNA is recognised and excised by cre. Tissue specific cre expression in transgenic mice enables the generation of tissue specific knock-out mice by mating gene targeted floxed mice with cre transgenic mice. Knock-out can be

conducted in every tissue (Schwenk et al., 1995) using the 'deleter' mouse or using transgenic mice with an inducible cre gene (such as those with tetracycline inducible cre genes), or knock-out can be tissue specific for example through the use of the CD19-cre mouse (Rickert et al., 1997).

According to still another aspect of the invention there is provided the use of genetically modified non-human animals for the screening of candidate pharmaceutical compounds.

It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country. Throughout this specification and the claims, the words "comprise", "comprises" and "comprising" are used in a non-exclusive sense, except where the context requires otherwise.

Brief Description of the Drawings

Figure 1. Nucleotide sequence of BNO627
Figure 2. Nucleotide sequence of BNO628
Figure 3. Nucleotide sequence of BNO629
Figure 4. Nucleotide sequence of BNO630
Figure 5. Nucleotide sequence of BNO631
Figure 6. Nucleotide sequence of BNO632
Figure 7. Nucleotide sequence of BNO633
Figure 8. Nucleotide sequence of BNO634
Figure 9. Nucleotide sequence of BNO636
Figure 10. Nucleotide sequence of BNO637
Figure 11. Nucleotide sequence of BNO638
Figure 12. Nucleotide sequence of BNO639
Figure 13. Nucleotide sequence of BNO640
Figure 14. Nucleotide sequence of BNO641
Figure 15. Nucleotide sequence of BNO928
Figure 16. Nucleotide sequence of BNO929

Figure 17. Nucleotide sequence of BNO930
Figure 18. Nucleotide sequence of BNO931
Figure 19. Nucleotide sequence of BNO932
Figure 20. Nucleotide sequence of BNO933
Figure 21. Nucleotide sequence of BNO934
Figure 22. Nucleotide sequence of BNO935
Figure 23. Nucleotide sequence of BNO936
Figure 24. Nucleotide sequence of BNO937
Figure 25. Nucleotide sequence of BNO938
Figure 26. Nucleotide sequence of BNO939
Figure 27. Nucleotide sequence of BNO940
Figure 28. Nucleotide sequence of BNO941
Figure 29. Nucleotide sequence of BNO942
Figure 30. Nucleotide sequence of BNO943
Figure 31. Nucleotide sequence of BNO944
Figure 32. Nucleotide sequence of BNO945
Figure 33. Nucleotide sequence of BNO946
Figure 34. Nucleotide sequence of BNO947
Figure 35. Nucleotide sequence of BNO948
Figure 36. Nucleotide sequence of BNO949
Figure 37. Nucleotide sequence of BNO950
Figure 38. Nucleotide sequence of BNO951
Figure 39. Nucleotide sequence of BNO952
Figure 40. Nucleotide sequence of BNO953
Figure 41. Nucleotide sequence of BNO954
Figure 42. Nucleotide sequence of BNO955
Figure 43. Nucleotide sequence of BNO956
Figure 44. Nucleotide sequence of BNO957

Figure 45. Example of the expression profile of selected differentially expressed clones during defined time points in the *in vitro* model of angiogenesis. Time points at the defined stages of 0.5 hours, 3 hours, 6 hours and 24 hours of the *in vitro* tube formation assay were plotted against the log ratio of cy5 (red) and cy3 (green) dyes used for microarray hybridisations. A: example of a clone with peak expression at the 0.5 hour

time point; B: example of a clone with peak expression at the 3 hour time point.

Figure 46. Example of the expression profile of selected differentially expressed clones during defined time points in the *in vitro* model of angiogenesis. Time points at the defined stages of 0.5 hours, 3 hours, 6 hours and 24 hours of the *in vitro* tube formation assay were plotted against the log ratio of cy5 (red) and cy3 (green) dyes used for microarray hybridisations. A: example of a clone with peak expression at the 6 hour time point; B: example of a clone with peak expression at the 24 hour time point.

Modes for Performing the Invention

Example 1: *In vitro* capillary tube formation

The *in vitro* model of angiogenesis is essentially as described in Gamble et al (1993). The assay was performed in collagen under the stimulation of phorbol myristate acetate (PMA) and the anti-integrin ($\alpha_2\beta_1$) antibody, RMACII. Human umbilical vein endothelial cells (HUVECs) were used in all experiments between passages 2 to 4.

Cells were harvested from bulk cultures (t=0), replated onto the collagen gels with stimulation and then harvested from the collagen gels at 0.5, 3.0, 6.0 and 24 hours after commencement of the assay. These time points were chosen since major morphological changes occur at these stages. Briefly, by 0.5 hours, cells have attached to the collagen matrix and have commenced migration into the gel. By 3.0 hours, small intracellular vesicles are visible. By 6.0 hours, these vesicles are coalescing together to form membrane bound vacuoles and the cells in the form of short sprouts have invaded the gel. After this time, these vacuoles fuse with the plasma membrane, thus expanding the intercellular space to generate the lumen (Meyer et al., 1997). The formation of these larger vacuoles is an essential requirement of lumen formation (Gamble et al., 1999). By 24 hours, the overall

anastomosing network of capillary tubes has formed and has commenced degeneration.

Example 2: RNA isolation, cDNA synthesis and amplification

Cells harvested at the specified time points were used for the isolation of total RNA using the Trizol reagent (Gibco BRL) according to manufacturers conditions. SMART (Switching mechanism at 5' end of RNA transcript) technology was used to convert small amounts of total RNA into enough cDNA to enable cDNA subtraction to be performed (see below). This was achieved using the SMART-PCR cDNA synthesis kit (Clontech-user manual PT3041-1) according to manufacturers recommendations. The SMART-PCR cDNA synthesis protocol generated a majority of full length cDNAs which were subsequently PCR amplified for cDNA subtraction.

Example 3: Suppression subtractive hybridisation (SSH)

SSH was performed on SMART amplified cDNA in order to enrich for cDNAs that were either up-regulated or down-regulated between the cDNA populations defined by the selected time-points. This technique also allowed "normalisation" of the regulated cDNAs, thereby making low abundance cDNAs (i.e. poorly expressed, but important, genes) more easily detectable. To do this, the PCR-Select cDNA synthesis kit (Clontech-user manual PT3041-1) and PCR-Select cDNA subtraction kit (Clontech-user manual PT1117-1) were used based on manufacturers conditions. These procedures relied on subtractive hybridisation and suppression PCR amplification. SSH was performed between the following populations: 0 - 0.5 hours; 0.5 - 3.0 hours; 3.0 - 6.0 hours; 6.0 - 24 hours.

Example 4: Differential screening of cDNA clones

Following SSH, the cDNA fragments were digested with *Eag*I and cloned into the compatible unique *Not*I site in pBluescript KS$^+$ using standard techniques (Sambrook et al.,

1989). This generated forward and reverse subtracted libraries for each time period. Initially, the forward subtracted libraries were used in subsequent studies to identify those clones representing genes that were up-regulated in their expression during the *in vitro* model of angiogenesis. To do this, a microarray analysis procedure was adopted.

Microarray slide preparation

A total of 10,000 clones from the 4 forward subtracted libraries (3,200 clones from 0-0.5 hr; 3,000 clones from 0.5-3 hr; 2,800 clones from 3-6 hr; 1,000 clones from 6-24 hr) were chosen to construct microarray slides. Inserts from these clones were amplified using standard PCR techniques with flanking T3 and T7 pBluescript KS$^+$ vector primers. DNA from each clone was spotted in duplicate onto a single microarray slide. Appropriate positive and negative controls were also incorporated onto the plate.

Probe labelling

Human umbilical vein endothelial cells harvested at the specified time points (0, 0.5, 3, 6, and 24 hr) were used for the isolation of total RNA using the Trizol reagent (Gibco BRL) according to manufacturers conditions. From each time point, 0.5 ug of total RNA was used as a template for the amplification of antisense RNA (aRNA) using the Ambion MessageAmp™ aRNA Kit. Briefly, total RNA was reversed transcribed with a T7 oligo(dT) primer in order to synthesize cDNA containing a T7 promoter sequence extending from the poly(A) tails of messages generated by reverse transcription. The cDNA was converted to a double-stranded DNA template and used for *in vitro* transcription of aRNA, incorporating 5-(3-aminoallyl)-UTP so as to allow coupling of fluorescent CyDyes. A typical amplification reaction would yield approximately 10 ug of mRNA (>400X

amplification, assuming the initial total RNA contained <5% mRNA).

Microarray hybridisation

After coupling of CyDyes, the synthesized aRNA was used as a probe (3.0-3.5 ug) for hybridisation to a microarray slide. The hybrizations performed were as follows:

1. 0 vs 0.5h (6 slides, 3 Dye swaps)
2. 0 vs 3h (4 slides, 2 Dye swaps)
3. 0 vs 6h (4 slides, 2 Dye swaps)
4. 0 vs 24h (4 slides, 2 Dye swaps)

Multiple slides were hybridized for each time point in order to verify the result from any one hybridization. Slides were hybridized in chambers for 16 hours, washed, and then scanned using the GenePix 2000 scanner. Those clones that were shown to be highly up-regulated were chosen for further analysis.

Example 5: Clone selection

From analysis of the microarray hybridizations, a total of 1,963 clones were identified to be up-regulated at specified time points during the *in vitro* model of angiogenesis. Figures 45 and 46 provide an example of the expression profiles observed during defined time points in the *in vitro* model for a selection of clones. Each of the 1,963 clones were sequenced and subsequent *in silico* database analysis was used to remove clones containing vector sequences only and clones for which poor sequence was obtained. Following this, redundancy screens were used to group clones according to individual genes that they represented. This left a total of 643 genes that were found to be up-regulated in their expression during the process of angiogenesis.

Table 1 provides information on the differentially expressed genes that were identified.

Example 6: Analysis of the angiogenic genes

The genes identified by this study to be implicated in the angiogenesis process, as listed in Table 1, may be used for further studies in order to confirm their role in angiogenesis *in vitro*. To do this, full-length coding sequences of the genes can be cloned into suitable expression vectors such as retroviruses or adenoviruses in both sense and anti-sense orientations and used for infection into endothelial cells (ECs). Retrovirus infection gives long-term EC lines expressing the gene of interest whereas adenovirus infection gives transient gene expression. Infected cells can then be subjected to a number of EC assays including those which measure proliferation and capillary tube formation to confirm the role of each gene in angiogenesis.

Protein interaction studies

The ability of any one of the angiogenic proteins of the invention to bind known and unknown proteins can be examined. Procedures such as the yeast two-hybrid system are used to discover and identify any functional partners. The principle behind the yeast two-hybrid procedure is that many eukaryotic transcriptional activators, including those in yeast, consist of two discrete modular domains. The first is a DNA-binding domain that binds to a specific promoter sequence and the second is an activation domain that directs the RNA polymerase II complex to transcribe the gene downstream of the DNA binding site. Both domains are required for transcriptional activation as neither domain can activate transcription on its own. In the yeast two-hybrid procedure, the gene of interest or parts thereof (BAIT), is cloned in such a way that it is expressed as a fusion to a peptide that has a DNA binding domain. A second gene, or number of genes, such as those from a cDNA library (TARGET), is cloned so that it is expressed as a fusion to an activation domain. Interaction of the protein of interest with its binding partner brings

the DNA-binding peptide together with the activation domain and initiates transcription of the reporter genes. The first reporter gene will select for yeast cells that contain interacting proteins (this reporter is usually a nutritional gene required for growth on selective media). The second reporter is used for confirmation and while being expressed in response to interacting proteins it is usually not required for growth.

The nature of the interacting genes and proteins can also be studied such that these partners can also be targets for drug discovery.

Structural studies

Recombinant angiogenic proteins of the invention can be produced in bacterial, yeast, insect and/or mammalian cells and used in crystallographical and NMR studies. Together with molecular modeling of the protein, structure-driven drug design can be facilitated.

TABLE 1

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO435	ICAM1	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	Hs.168383	NM_000201	3
BNO436	NP	nucleoside phosphorylase	Hs.75514	NM_000270	6
BNO437	IL8	interleukin 8	Hs.624	NM_000584	3
BNO438	CD59	CD59 antigen p18-20	Hs.278573	NM_000611	24
BNO439	VCAM1	vascular cell adhesion molecule 1	Hs.109225	NM_001078	3
BNO440	ANGPT2	angiopoietin 2	Hs.115181	NM_001147	6
BNO441	BIRC3	baculoviral IAP repeat-containing 3	Hs.127799	NM_001165	3
BNO442	FABP5	fatty acid binding protein 5 (psoriasis-associated)	Hs.408061	NM_001444	24
BNO443	CBFB	core-binding factor, beta subunit	Hs.179881	NM_001755	6
BNO444	CTNNB1	catenin (cadherin-associated protein), beta 1, 88kDa	Hs.171271	NM_001904	3
BNO445	F3	coagulation factor III (thromboplastin, tissue factor)	Hs.62192	NM_001993	3
BNO446	INHBA	inhibin, beta A (activin A, activin AB alpha polypeptide)	Hs.727	NM_002192	6
BNO447	MGST2	microsomal glutathione S-transferase 2	Hs.81874	NM_002413	24
BNO448	RAB6A	RAB6A, member RAS oncogene family	Hs.5636	NM_002869	6
BNO449	SAT	spermidine/spermine N1-acetyltransferase	Hs.28491	NM_002970	6
BNO450	STC1	stanniocalcin 1	Hs.25590	NM_003155	24
BNO451	TXNRD1	thioredoxin reductase 1	Hs.13046	NM_003330	6
BNO452	SLC4A7	solute carrier family 4, sodium bicarbonate cotransporter, member 7	Hs.132904	NM_003615	6
BNO453	PPAP2B	phosphatidic acid phosphatase type 2B	Hs.432840	NM_003713	3
BNO454	BCL10	B-cell CLL/lymphoma 10	Hs.193516	NM_003921	3
BNO455	DUSP1	dual specificity phosphatase 1	Hs.171695	NM_004417	0.5
BNO456	KIF5B	kinesin family member 5B	Hs.149436	NM_004521	6
BNO457	WTAP	Wilms' tumour 1-associating protein	Hs.119	NM_004906	0.5
BNO458	ADAMTS4	a disintegrin-like and metalloprotease (thrombospondin type 1 motif, 4)	Hs.211604	NM_005099	6
BNO459	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	Hs.25647	NM_005252	0.5
BNO460	GATA6	GATA binding protein 6	Hs.50924	NM_005257	3
BNO461	HRY	hairy and enhancer of split 1, (Drosophila)	Hs.250666	NM_005524	0.5
BNO462	SGK	serum/glucocorticoid regulated kinase	Hs.296323	NM_005627	3

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO463	TIEG	TGFB inducible early growth response	Hs.82173	NM_005655	0.5
BNO464	DXS1357E	accessory protein BAP31	Hs.291904	NM_005745	
BNO465	CALCRL	calcitonin receptor-like	Hs.152175	NM_005795	24
BNO466	SUI1	putative translation initiation factor	Hs.150580	NM_005801	3
BNO467	TSC22	transforming growth factor beta-stimulated protein TSC-22	Hs.114360	NM_006022	6
BNO468	RAN	RAN, member RAS oncogene family	Hs.426035	NM_006325	
BNO469	LYPLA1	lysophospholipase I	Hs.12540	NM_006330	6
BNO470	SSFA2	sperm specific antigen 2	Hs.351355	NM_006751	6
BNO471	ESM1	endothelial cell-specific molecule 1	Hs.41716	NM_007036	3, 24
BNO472	CLIC4	chloride intracellular channel 4	Hs.25035	NM_013943	24
BNO473	SLC7A11	solute carrier family 7, member 11	Hs.6682	NM_014331	3
BNO474	RAI14	retinoic acid induced 14	Hs.15165	NM_015577	6
BNO475	HSPC014	chromosome 13 open reading frame 12	Hs.279813	NM_015932	24
BNO476	UMP-CMPK	UMP-CMP kinase	Hs.11463	NM_016308	3
BNO477	SLC38A2	solute carrier family 38, member 2	Hs.298275	NM_018976	3
BNO478	ZNF317	zinc finger protein 317	Hs.18587	NM_020933	24
BNO479	RAB6C	RAB6C, member RAS oncogene family	Hs.333139	NM_032144	24
BNO480	MKI67IP	MKI67 (FHA domain) interacting nucleolar phosphoprotein	Hs.142838	NM_032390	3
BNO481	KPNA4	karyopherin alpha 4 (importin alpha 3)	Hs.288193	NM_002268	3
BNO482	CMG2	capillary morphogenesis protein 2	Hs.5897	NM_058172	6
BNO483	C14orf32	chromosome 14 open reading frame 32	Hs.406401	NM_144578	3
BNO484	SMARCA2	SWI/SNF related, matrix associated, regulator of chromatin, A2	Hs.198296	NM_003070	0.5
BNO485	SOX4	Homo sapiens SRY (sex determining region Y)-box 4 (SOX4), mRNA	Hs.83484	NM_003107	3
BNO486	EFNB2	ephrin-B2	Hs.30942	NM_004093	3
BNO487	NR4A3	nuclear receptor subfamily 4, group A, member 3	Hs.80561	NM_006981	0.5
BNO488	NTN4	netrin 4	Hs.102541	NM_021229	
BNO489	DNCI2	dynein, cytoplasmic, intermediate polypeptide 2 (DNCI2), mRNA	Hs.66881	XM_027780	0.5

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO490	UGCG	UDP-glucose ceramide glucosyltransferase	Hs.432605	NM_003358	0.5, 24
BNO491	P125	Sec23-interacting protein p125	Hs.300208	NM_007190	3
BNO492	NUDT4	nudix (nucleoside diphosphate linked moiety X)-type motif 4	Hs.355399	NM_019094	6
BNO493	PTGS1	prostaglandin-endoperoxide synthase 1	Hs.88474	NM_000962	6
BNO494	KDR	kinase insert domain receptor (a type III receptor tyrosine kinase)	Hs.12337	NM_002253	
BNO495	SATB1	special AT-rich sequence binding protein 1	Hs.74592	NM_002971	6
BNO496	BZW1	basic leucine zipper and W2 domains 1	Hs.155291	NM_014670	3
BNO497	TDG	thymine-DNA glycosylase	Hs.173824	NM_003211	6
BNO498	ACTR3	ARP3 actin-related protein 3 homolog (yeast)	Hs.380096	NM_005721	24
BNO499	LAMP2	lysosomal-associated membrane protein 2	Hs.8262	NM_013995	6
BNO500	ERBB2IP	erbb2 interacting protein	Hs.8117	NM_018695	6
BNO501	DNAJB6	DnaJ (Hsp40) homolog, subfamily B, member 6	Hs.181195	NM_005494	3
BNO502	EMP1	epithelial membrane protein 1	Hs.79368	NM_001423	6
BNO503	MAPK1	mitogen-activated protein kinase 1	Hs.324473	NM_002745	24
BNO504	CYP1A1	cytochrome P450, subfamily 1, polypeptide 1	Hs.72912	NM_000499	6
BNO505	ACVR1	activin A receptor, type I	Hs.150402	NM_001105	3
BNO506	TPT1	tumor protein, translationally-controlled 1	Hs.401448	NM_003295	0.5, 24
BNO507	VAV3	vav 3 oncogene	Hs.267659	NM_006113	3
BNO508	CAP	adenylyl cyclase-associated protein	Hs.104125	NM_006367	24
BNO509	HSPA5	Heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)	Hs.75410	NM_005347	6
BNO510	TIA1	TIA1 cytotoxic granule-associated RNA binding protein	Hs.239489	NM_022173	6
BNO511	CCNT2	cyclin T2	Hs.155478	NM_001241	6
BNO512	CHC1L	chromosome condensation 1-like	Hs.27007	NM_001268	0.5
BNO513	SFPQ	splicing factor proline/glutamine rich	Hs.180610	NM_005066	3
BNO514	PRKAR1A	protein kinase, cAMP-dependent, regulatory, type I, alpha	Hs.183037	NM_002734	24
BNO515	RALA	v-ral simian leukemia viral oncogene homolog A (ras related)	Hs.6906	NM_005402	6
BNO516	ANXA2	annexin A2	Hs.217493	NM_004039	0.5
BNO517	NUP153	nucleoporin 153kDa	Hs.211608	NM_005124	3

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO518	RANBP9	RAN binding protein 9	Hs.279886	NM_005493	24
BNO519	PRPF4B	PRP4 pre-mRNA processing factor 4 homolog B (yeast)	Hs.198891	NM_003913	6
BNO520	TSN	translin	Hs.75066	NM_004622	6
BNO521	H3F3A	H3 histone, family 3A	Hs.181307	NM_002107	24
BNO522	F2R	coagulation factor II (thrombin) receptor	Hs.128087	NM_001992	3
BNO523	PROS1	protein S (alpha)	Hs.64016	NM_000313	6
BNO524	DDX3	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3	Hs.380774	NM_001356	3
BNO525	TCF4	transcription factor 4	Hs.326198	NM_003199	6
BNO526	PTP4A1	Protein tyrosine phosphatase type IVA, member 1	Hs.227777	NM_003463	6
BNO527	BMPR2	bone morphogenetic protein receptor, type II (serine/threonine kinase)	Hs.53250	NM_001204	3
BNO528	NFE2L2	nuclear factor (erythroid-derived 2)-like 2	Hs.155396	NM_006164	3
BNO529	CTSB	cathepsin B	Hs.297939	NM_001908	24
BNO530	LIF	leukemia inhibitory factor (cholinergic differentiation factor)	Hs.2250	NM_002309	3
BNO531	AHR	aryl hydrocarbon receptor	Hs.170087	NM_001621	3
BNO532	RANBP7	RAN binding protein 7	Hs.5151	NM_006391	3
BNO533	ARF6	ADP-ribosylation factor 6	Hs.89474	NM_001663	3
BNO534	SCARF1	SCARF1 Scavenger receptor class F, member 1	Hs.57735	NM_003693Ê	24
BNO535	PLU-1	putative DNA/chromatin binding motif	Hs.143323	NM_006618	24
BNO536	TOMM20	translocase of outer mitochondrial membrane 20 (yeast) homolog	Hs.75187	NM_014765	6
BNO537	B2M	beta-2-microglobulin	Hs.48516	NM_004048	24
BNO538	zizimin1	zizimin1	Hs.8021	NM_015296	6
BNO539	ARPP-19	cyclic AMP phosphoprotein, 19 kD	Hs.7351	NM_006628	3
BNO540	RAP1B	RAP1B, member of RAS oncogene family	Hs.156764	NM_015646	3
BNO541	MCP	membrane cofactor protein	Hs.83532	NM_153826	6
BNO542	IFI16	interferon, gamma-inducible protein 16	Hs.155530	NM_005531	0.5
BNO543	PRG1	proteoglycan 1, secretory granule	Hs.1908	NM_002727	
BNO544	KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	Hs.81665	NM_000222	0.5, 24
BNO545	SYBL1	synaptobrevin-like 1	Hs.24167	NM_005638	6

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO546	TCF8	transcription factor 8 (represses interleukin 2 expression)	Hs.232068	NM_030751Ê	6
BNO547	EDN1	endothelin 1	Hs.2271	NM_001955Ê	0.5
BNO548	NXF1	nuclear RNA export factor 1	Hs.323502	NM_006362	3, 24
BNO549	RAP2B	RAP2B, member of RAS oncogene family	Hs.239527	NM_002886	3
BNO550	JAK1	Janus kinase 1 (a protein tyrosine kinase)	Hs.50651	NM_002227	24
BNO551	IL6ST	interleukin 6 signal transducer (gp130, oncostatin M receptor)	Hs.82065	NM_002184	6
BNO552	REST	RE1-silencing transcription factor	Hs.401145	NM_005612	6
BNO553	SLC19A2	solute carrier family 19 (thiamine transporter), member 2	Hs.30246	NM_006996	3
BNO554	EIF4G2	eukaryotic translation initiation factor 4 gamma, 2	Hs.183684	NM_001418	3
BNO555	PTPRE	protein tyrosine phosphatase, receptor type, E	Hs.31137	NM_006504	3
BNO556	PDE3A	phosphodiesterase 3A, cGMP-inhibited	Hs.777	NM_000921	3
BNO557	C1QR1	complement component 1, q subcomponent, receptor 1	Hs.97199	NM_012072	24
BNO558	RANBP2	RAN binding protein 2	Hs.199179	NM_006267	
BNO559	KIS	kinase interacting with leukemia-associated gene (stathmin)	Hs.127310	NM_144624	24
BNO560	HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	Hs.11899	NM_000859	6
BNO561	PDCD4	programmed cell death 4 (neoplastic transformation inhibitor)	Hs.326248	NM_145341	3
BNO562	TACC1	transforming, acidic coiled-coil containing protein 1	Hs.173159	NM_006283	0.5
BNO563	THBD	thrombomodulin	Hs.2030	NM_000361	24
BNO564	DIS3	mitotic control protein dis3 homolog	Hs.323346	NM_014953	6
BNO565	TOP2A	topoisomerase (DNA) II alpha 170kDa	Hs.156346	NM_001067	6
BNO566	SLC7A2	solute carrier family 7, member 2	Hs.153985	NM_003046	6
BNO567	FH	fumarate hydratase	Hs.75653	NM_000143	6
BNO568	IL1RL1	interleukin 1 receptor-like 1	Hs.66	NM_003856	6
BNO569	HPRP3P	U4/U6-associated RNA splicing factor	Hs.11776	NM_004698	6
BNO570	DDX5	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5	Hs.76053	NM_004396	
BNO571	MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	Hs.79078	NM_002358	0.5, 24
BNO572	MADH7	MAD, mothers against decapentaplegic homolog 7 (Drosophila)	Hs.100602	NM_005904	3
BNO573	E2F3	E2F transcription factor 3	Hs.1189	NM_001949	3

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO574	CSNK2A2	CSNK2A2 Casein kinase 2, alpha prime polypeptide	Hs.82201	NM_001896	6
BNO575	MAX	MAX protein	Hs.42712	NM_002382	6
BNO576	ERAP140	140 kDa estrogen receptor associated protein	Hs.339283	AF493978	3
BNO577	CD9	CD9 antigen (p24)	Hs.1244	NM_001769	24
BNO578	ATRX	alpha thalassemia/mental retardation syndrome X-linked	Hs.96264	NM_000489	6
BNO579	YWHAZ	tyrosine/tryptophan activation protein, zeta polypeptide	Hs.75103	NM_003406	3
BNO580	IDS	iduronate 2-sulfatase (Hunter syndrome)	Hs.172458	NM_000202	24
BNO581	SERPINE2	serine (or cysteine) proteinase inhibitor, clade E, member 2	Hs.21858	NM_006216	6
BNO582	DDEF1	development and differentiation enhancing factor 1	Hs.10669	NM_018482	6
BNO583	GLRX	glutaredoxin (thioltransferase)	Hs.28988	NM_002064	24
BNO584	MAP3K1	MAP3K1 Mitogen-activated protein kinase kinase kinase 1	Hs.401150	AF042838	3
BNO585	ANKH	ankylosis, progressive homolog (mouse)	Hs.168640	NM_054027	3
BNO586	RBX1	ring-box 1	Hs.279919	NM_014248	24
BNO587	NAB1	NGFI-A binding protein 1 (EGR1 binding protein 1)	Hs.107474	NM_005966	3
BNO588	TNFSF10	tumor necrosis factor (ligand) superfamily, member 10	Hs.83429	NM_003810	3
BNO589	PRDX3	peroxiredoxin 3	Hs.75454	NM_006793	6
BNO590	MAP2K1	mitogen-activated protein kinase kinase 1	Hs.3446	NM_002755	3
BNO591	NFATC1	nuclear factor of activated T-cells, calcineurin-dependent 1	Hs.96149	NM_006162	24
BNO592	PSEN1	presenilin 1 (Alzheimer disease 3)	Hs.3260	NM_000021	0.5
BNO593	STAT3	signal transducer and activator of transcription 3	Hs.321677	NM_139276	6
BNO594	USP7	ubiquitin specific protease 7 (herpes virus-associated)	Hs.78683	NM_003470	
BNO595	ARHB	ras homolog gene family, member B	Hs.406064	NM_004040	3
BNO596	PTEN	phosphatase and tensin homolog	Hs.10712	NM_000314	
BNO597	UBL1	ubiquitin-like 1 (sentrin)	Hs.81424	NM_003352	24
BNO598	RAB5A	RAB5A, member RAS oncogene family	Hs.73957	NM_004162	3
BNO599	ITGB1	integrin, beta 1	Hs.287797	NM_002211	24
BNO600	PRDM2	PR domain containing 2, with ZNF domain	Hs.26719	NM_012231	6
BNO601	GJA1	gap junction protein, alpha 1, 43kDa (connexin 43)	Hs.74471	NM_000165	3

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO602	ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	Hs.271986	NM_002203	6
BNO603	ETV5	ets variant gene 5 (ets-related molecule)	Hs.43697	NM_004454	3
BNO604	ZFHX1B	zinc finger homeobox 1b	Hs.34871	NM_014795	3
BNO605	BNO605	EST, similar to JC1169 DNA-damage-inducible protein GADD153	Hs.335776	AW576601	
BNO606	LOC157713	lysophospholipase I-like pseudogene on chromosome 6		NG_001063	
BNO607	. RBM3	RNA binding motif protein 3	Hs.301404	NM_006743	0.5
BNO608	HEY1	hairy/enhancer-of-split related with YRPW motif 1	Hs.234434	NM_012258	0.5
BNO609	NET-6	transmembrane 4 superfamily member tetraspan NET-6	Hs.284243	NM_014399	6
BNO610	EHD3	EH-domain containing 3	Hs.87125	NM_014600	24
BNO611	KIAA0992	palladin	Hs.194431	NM_016081	6
BNO612	FLJ20445	hypothetical protein FLJ20445	Hs.343748	NM_017824	6
BNO613	METL	methyltransferase like 2	Hs.433213	NM_018396	3
BNO614	HT010	uncharacterized hypothalamus protein HT010	Hs.6375	NM_018471	0.5
BNO615	C3orf4	chromosome 3 open reading frame 4	Hs.107393	NM_019895	6
BNO616	MGC2747	hypothetical protein MGC2747	Hs.194017	NM_024104	
BNO617	FLJ20986	hypothetical protein FLJ20986	Hs.324507	NM_024524	6
BNO618	FLJ14834	hypothetical protein FLJ14834	Hs.62905	NM_032849	3
BNO619	RPL27A	ribosomal protein L27a	Hs.76064	NM_000990	6
BNO620	FLJ22746	hypothetical protein FLJ22746	Hs.147585	NM_024785	0.5
BNO621	KIAA1323	KIAA1323 protein	Hs.34892	XM_032146	0.5
BNO622	KIAA1376	KIAA1376 protein	Hs.24684	XM_033042	3, 24
BNO623	KIAA0261	KIAA0261 protein	Hs.154978	XM_042946	24
BNO624	KIAA1199	KIAA1199 protein	Hs.50081	XM_051860	6
BNO625	HIF1	huntingtin interacting protein B	Hs.6947	NM_014159	
BNO626	LOC221634	full length insert cDNA clone EUROIMAGE 1977059	Hs.351928	AJ420500	3
BNO627	BNO627	EST		Figure 1	6
BNO628	BNO628	EST		Figure 2	6
BNO629	BNO629	EST		Figure 3	6

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO630	BNO630	EST		Figure 4	6
BNO631	BNO631	ESTs, Weakly similar to neuronal thread protein	Hs.172998	Figure 5	24
BNO632	BNO632	ESTs	Hs.404198	Figure 6	6
BNO633	BNO633	ESTs, Weakly similar to hypothetical protein FLJ20378	Hs.310598	Figure 7	24
BNO634	BNO634	ESTs	Hs.345443	Figure 8	6
BNO635	BNO635	Homo sapiens cDNA: FLJ21191 fis, clone COL00104	Hs.54347	AK024844	6
BNO636	BNO636	ESTs, Highly similar to putative protein-tyrosine kinase	Hs.105636	Figure 9	3
BNO637	BNO637	ESTs		Figure 10	6
BNO638	BNO638	contains Alu repetitive element		Figure 11	6
BNO639	BNO639	NONE		Figure 12	6
BNO640	BNO640	NONE		Figure 13	6
BNO641	BNO641	ESTs	Hs.406588	Figure 14	3
BNO642	ETL	EGF-TM7-latrophilin-related protein	Hs.57958	NM_022159	24
BNO643	VMP1	likely ortholog of rat vacuole membrane protein 1	Hs.166254	NM_030938	3
BNO644	TAF9	TATA box binding protein (TBP)-associated factor, 32kDa	Hs.60679	NM_016283	24
BNO645	FLJ10498	hypothetical protein FLJ10498	Hs.109045	NM_018115	24
BNO646	MAN1A1	mannosidase, alpha, class 1A, member 1	Hs.432931	NM_005907	6
BNO647	KIAA0716	KIAA0716 gene product	Hs.118140	NM_014705	24
BNO648	LOC57146	hypothetical protein from clone 24796	Hs.27191	NM_020422	0.5
BNO649	ADAMTS9	a disintegrin-like and metalloprotease (thrombospondin type 1 motif, 9)	Hs.126855	NM_020249	24
BNO650	BNO650	Unnamed protein product	Hs.378718	AK090752	6
BNO651	RPLP0	ribosomal protein, large, P0	Hs.406511	NM_001002	6
BNO652	FLJ31051	hypothetical protein FLJ31051	Hs.406199	NM_153687	6
BNO653	GALNT4	N-acetylgalactosaminyltransferase 4	Hs.271923	NM_003774	3
BNO654	GNG2	guanine nucleotide binding protein (G protein), gamma 2	Hs.289026	BC020774	6
BNO655	LOC51122	HSPC042 protein	Hs.265540	NM_016094	3
BNO656	MBNL	muscleblind-like (Drosophila)	Hs.28578	NM_021038	
BNO657	BNO657	cDNA DKFZp434O1317	Hs.25362	AL133611	3

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO658	ASB3	ankyrin repeat and SOCS box-containing 3	Hs.9893	NM_016115	6
BNO659	FLJ32123	FLJ32123	Hs.349397	AK056685	6
BNO660	GG2-1	TNF-induced protein	Hs.17839	NM_014350	3
BNO661	ELL2	ELL-related RNA polymerase II, elongation factor	Hs.98124	NM_012081	3
BNO662	BNO662	ESTs	Hs.209356	AL043805	6
BNO663	ATP5J2	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit f 2	Hs.235557	NM_004889	24
BNO664	FLJ10312	FLJ10312	Hs.132560	NM_030672	3
BNO665	SDCBP	syndecan binding protein (syntenin)	Hs.8180	NM_005625	3
BNO666	KIAA1959	KIAA1959 Nm23-phosphorylated unknown substrate	Hs.55067	NM_032873	3
BNO667	GNPNAT1	glucosamine-phosphate N-acetyltransferase 1	Hs.27931	AK090577	6
BNO668	FLJ33903	hypothetical protein FLJ33903	Hs.302718	NM_152594	
BNO669	BNO669	ESTs, Weakly similar to neuronal thread protein	Hs.164588	BC030094	3
BNO670	Nbak2	homeodomain interacting protein kinase 1-like protein	Hs.12259	NM_152696	6
BNO671	KIAA0882	KIAA0882 protein	Hs.90419	AB020689	3
BNO672	GABPA	GA binding protein transcription factor, alpha subunit 60kDa	Hs.78	NM_002040	3
BNO673	BNO673	hypothetical protein DKFZp434L142	Hs.323583	NM_016613	6
BNO674	V-1	likely ortholog of rat V-1 protein	Hs.21321	NM_145808	24
BNO675	FLJ10700	hypothetical protein FLJ10700	Hs.295909	NM_018182	3
BNO676	C8FW	phosphoprotein regulated by mitogenic pathways	Hs.7837	NM_025195	3
BNO677	FLJ30135	FLJ30135	Hs.34906	BC020494	3, 24
BNO678	TBC1D4	TBC1 domain family, member 4	Hs.173802	NM_014832	6
BNO679	ACATE2	likely ortholog of mouse acyl-Coenzyme A thioesterase 2	Hs.18625	NM_012332	24
BNO680	CRYZ	crystallin, zeta (quinone reductase)	Hs.83114	NM_001889	6
BNO681	KPNB1	karyopherin (importin) beta 1	Hs.180446	NM_002265	24
BNO682	RPL23A	ribosomal protein L23a	Hs.350046	NM_000984	0.5
BNO683	LIMS1	LIM and senescent cell antigen-like domains 1	Hs.112378	NM_004987	6
BNO684	WW45	WW45 protein	Hs.288906	NM_021818	3
BNO685	FLJ10849	hypothetical protein FLJ10849	Hs.8768	NM_018243	

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO686	ST3GALVI	alpha2,3-sialyltransferase	Hs.34578	NM_006100	6
BNO687	MGC45416	hypothetical protein MGC45416	Hs.95835	NM_152398	24
BNO688	CPR8	cell cycle progression 8 protein	Hs.283753	NM_004748	24
BNO689	HDCL	hHDC for homolog of Drosophila headcase	Hs.6679	NM_016217	3
BNO690	C15orf15	chromosome 15 open reading frame 15	Hs.284162	NM_016304	3
BNO691	UBC	ubiquitin C	Hs.183704	NM_021009	3
BNO692	RDX	radixin	Hs.263671	NM_002906	24
BNO693	PELI1	pellino homolog 1 (Drosophila)	Hs.7886	NM_020651	3
BNO694	BNO694	cDNA DKFZp566E0124	Hs.401218	AL050030	6
BNO695	MCC	mutated in colorectal cancers	Hs.1345	NM_002387	6
BNO696	RetSDR2	RetSDR2 Retinal short-chain dehydrogenase/reductase 2	Hs.12150	NM_016245	3
BNO697	BNO697	Unknown (protein for IMAGE:4564684)	Hs.345588	BC014203	24
BNO698	BNO698	ESTs, Weakly similar to carbohydrate (chondroitin) synthase 1	Hs.165050	BE747231	3
BNO699	BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)	Hs.80426	NM_004899	6
BNO700	LOC115416	hypothetical protein BC012331	Hs.87385	NM_138446	24
BNO701	BAZ1A	bromodomain adjacent to zinc finger domain, 1A	Hs.8858	NM_013448	3
BNO702	HNRPDL	heterogeneous nuclear ribonucleoprotein D-like	Hs.372673	NM_005463	3
BNO703	PREI3	preimplantation protein 3	Hs.107942	NM_015387	6
BNO704	KIAA1102	KIAA1102 protein	Hs.202949	AB029025	
BNO705	BNO705	ESTs	Hs.30280	BG121629	3
BNO706	LOC116441	hypothetical protein BC014339	Hs.22026	NM_138786	24
BNO707	BNO707	Human XIST, coding sequence "a"	Hs.352403	X56199	3
BNO708	BNO708	ESTs	Hs.12876	BM991801	6
BNO709	ROD1	ROD1 regulator of differentiation 1 (S. pombe)	Hs.374634	NM_005156	6
BNO710	BNO710	FLJ23228	Hs.114121	AK026881	6
BNO711	SMAP-5	golgi membrane protein SB140	Hs.5672	NM_030799	6
BNO712	BNO712	FLJ21592	Hs.5921	AK025245	3
BNO713	KIAA0970	KIAA0970 protein	Hs.103329	NM_014923	6

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO714	KIAA0121	KIAA0121 gene product	Hs.155584	D50911	6
BNO715	KIAA0864	KIAA0864 KIAA0864 protein	Hs.433523	AB020671	0.5, 24
BNO716	HIVEP2	human immunodeficiency virus type I enhancer binding protein 2	Hs.75063	NM_006734	3
BNO717	DC42	hypothetical protein DC42	Hs.72805	NM_030921	3
BNO718	FLJ33918	hypothetical protein FLJ33918	Hs.17121	NM_152407	6
BNO719	PCMF	potassium channel modulatory factor	Hs.5392	NM_020122	3
BNO720	UBE2E1	ubiquitin-conjugating enzyme E2E 1 (UBC4/5 homolog, yeast)	Hs.163546	NM_003341	24
BNO721	KLHL4	kelch-like 4 (Drosophila)	Hs.49075	NM_019117	
BNO722	FLJ12838	mandaselin	Hs.46903	NM_024641	3
BNO723	HSPC134	HSPC134 protein	Hs.279761	NM_014169	6
BNO724	TCF12	transcription factor 12 (HTF4, helix-loop-helix transcription factors 4)	Hs.21704	NM_003205	6
BNO725	KIAA0582	KIAA0582 protein	Hs.79507	NM_015147	24
BNO726	STAF42	SPT3-associated factor 42	Hs.107968	NM_053053	6
BNO727	CYFIP1	cytoplasmic FMR1 interacting protein 1	Hs.77257	NM_014608	6
BNO728	NOL5A	nucleolar protein 5A (56kDa with KKE/D repeat)	Hs.296585	NM_006392	6
BNO729	GSA7	ubiquitin activating enzyme E1-like protein	Hs.278607	NM_006395	6
BNO730	BNO730	ESTs	Hs.158753	BG539522	6
BNO731	FLJ10342	hypothetical protein FLJ10342	Hs.101514	NM_018064	3
BNO732	FLJ20085	hypothetical protein FLJ20085	Hs.118964	NM_017660	
BNO733	STAG1	stromal antigen 1	Hs.286148	NM_005862	
BNO734	FLJ21269	hypothetical protein FLJ21269	Hs.18160	NM_025107	6
BNO735	FLJ32029	Unnamed protein product	Hs.26612	NM_173582	6
BNO736	SCAMP1	secretory carrier membrane protein 1	Hs.31218	NM_004866	3
BNO737	BNO737	hypothetical protein DKFZp434F0318	Hs.23388	NM_030817	
BNO738	ACTG1	actin, gamma 1	Hs.14376	NM_001614	0.5
BNO739	HRB2	HIV-1 rev binding protein 2	Hs.154762	NM_007043	6
BNO740	KIAA1728	KIAA1728 protein	Hs.252748	AB051515	24
BNO741	BNO741	ESTs	Hs.374415	BM803108	6

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO742	BNO742	ESTs, Weakly similar to hypothetical protein MGC5540	Hs.99496	BG183686	24
BNO743	BCAT1	branched chain aminotransferase 1, cytosolic	Hs.317432	NM_005504	0.5, 24
BNO744	KIAA0438	KIAA0438 gene product	Hs.279849	NM_014819	
BNO745	BNO745	hypothetical protein DKFZp547A023	Hs.23921	NM_018704	6
BNO746	FKSG14	leucine zipper protein FKSG14	Hs.192843	NM_022145	6
BNO747	MGC23937	hypothetical protein MGC23937 similar to CG4798	Hs.91612	NM_145052	6
BNO748	KLHL6	kelch-like 6 (Drosophila)	Hs.43616	NM_130446	6
BNO749	MGC46235	hypothetical protein MGC46235	Hs.6127	NM_153712	6
BNO750	CDC23	CDC23 (cell division cycle 23, yeast, homolog)	Hs.153546	NM_004661	24
BNO751	ULK2	unc-51-like kinase 2 (C. elegans)	Hs.151406	NM_014683	3
BNO752	SCARB2	SCARB2 Scavenger receptor class B, member 2	Hs.323567	NM_005506	3
BNO753	BNO753	cDNA DKFZp667P1024	Hs.201008	AL832835	3
BNO754	KIAA0303	KIAA0303 protein	Hs.432631	AB002301	3
BNO755	ZMPSTE24	zinc metalloproteinase (STE24 homolog, yeast)	Hs.25846	NM_005857	
BNO756	BNO756	ESTs, Weakly similar to hypothetical protein FLJ20489	Hs.117269	BM720565	
BNO757	U5-100K	prp28, U5 snRNP 100 kd protein	Hs.184771	NM_004818	
BNO758	CHD4	chromodomain helicase DNA binding protein 4	Hs.74441	NM_001273	6
BNO759	KIAA1416	KIAA1416 protein	Hs.105461	AB037837	6
BNO760	CGI-127	yippee protein	Hs.184542	NM_016061	3, 24
BNO761	MGC3077	hypothetical protein MGC3077	Hs.433404	NM_024051	6
BNO762	FLJ11223	cDNA FLJ11223	Hs.92308	AL832083	3
BNO763	BET1	BET1 homolog (S. cerevisiae)	Hs.23103	NM_005868	24
BNO764	ARHGAP5	Rho GTPase activating protein 5	Hs.267831	NM_001173	
BNO765	KIAA1010	KIAA1010 protein	Hs.23860	AB023227	3
BNO766	NUMB	numb homolog (Drosophila)	Hs.78890	NM_003744	6
BNO767	P5	protein disulfide isomerase-related protein	Hs.182429	NM_005742	0.5
BNO768	FLJ30478	cDNA FLJ30478	Hs.298258	AK092048	6
BNO769	SFRS2IP	splicing factor, arginine/serine-rich 2, interacting protein	Hs.51957	NM_004719	6

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO770	OXA1L	oxidase (cytochrome c) assembly 1-like	Hs.151134	NM_005015	0.5, 24
BNO771	POH1	26S proteasome-associated pad1 homolog	Hs.178761	NM_005805	6
BNO772	FLJ10004	cDNA FLJ10004	Hs.381207	AK095297	6
BNO773	AHCYL1	S-adenosylhomocysteine hydrolase-like 1	Hs.4113	NM_006621	3
BNO774	UAP1	UDP-N-acteylglucosamine pyrophosphorylase 1	Hs.21293	NM_003115	3
BNO775	PLS3	plastin 3 (T isoform)	Hs.4114	NM_005032	6
BNO776	TSNAX	translin-associated factor X	Hs.96247	NM_005999	0.5
BNO777	HELO1	homolog of yeast long chain polyunsaturated fatty acid elong. enz. 2	Hs.250175	NM_021814	6
BNO778	MAN2A1	mannosidase, alpha, class 2A, member 1	Hs.377915	NM_002372	3
BNO779	RAB21	RAB21, member RAS oncogene family	Hs.184627	NM_014999	6
BNO780	LOC58489	hypothetical protein from EUROIMAGE 588495	Hs.26765	AL390079	3
BNO781	WAC	WW domain-containing adapter with a coiled-coil region	Hs.70333	NM_016628	3
BNO782	MGC26717	Similar to RIKEN cDNA 4930453N24 gene	Hs.406060	BC024188	6
BNO783	POSH	likely ortholog of mouse plenty of SH3 domains	Hs.301804	AB040927	6
BNO784	RBM9	RNA binding motif protein 9	Hs.351478	NM_014309	
BNO785	CSRP2	cysteine and glycine-rich protein 2	Hs.10526	NM_001321	3
BNO786	COPA	coatomer protein complex, subunit alpha	Hs.75887	NM_004371	6
BNO787	TIMM17A	translocase of inner mitochondrial membrane 17 homolog A (yeast)	Hs.20716	NM_006335	6
BNO788	RIN2	Ras and Rab interactor 2	Hs.62349	NM_018993	24
BNO789	KLHL5	kelch-like 5 (Drosophila)	Hs.272239	NM_015990	24
BNO790	IPLA2(γ)	intracellular memb.-assoc. calcium-independent phospholipase A2 γ	Hs.44198	AF263613	6
BNO791	KIAA1053	KIAA1053 protein	Hs.173571	NM_015589	6
BNO792	BNO792	ESTs, Highly similar to M3K1	Hs.170610	BF510609	
BNO793	KIAA0766	KIAA0766 gene product	Hs.28020	NM_014805	24
BNO794	SMARCA5	SWI/SNF related regulator of chromatin, a5	Hs.9456	NM_003601	
BNO795	BNO795	ESTs, Weakly similar to sarcosine dehydrogenase	Hs.199426	BF526552	6
BNO796	FBXL3A	F-box and leucine-rich repeat protein 3A	Hs.7540	NM_012158	24
BNO797	SART2	squamous cell carcinoma antigen recognized by T cell	Hs.58636	NM_013352Ê	6

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO798	YH66D08	Homo sapiens full length insert cDNA YH66D08, mRNA sequence	Hs.71848	U79277	
BNO799	SH3BGRL2	SH3 domain binding glutamic acid-rich protein like 2	Hs.9167	NM_031469	3, 24
BNO800	KIAA1577	KIAA1577 protein	Hs.13913	AB046797	6
BNO801	PUM1	pumilio homolog 1 (Drosophila)	Hs.153834	NM_014676	3
BNO802	KIAA0877	KIAA0877 protein	Hs.11217	AB020684	24
BNO803	CCT2	chaperonin containing TCP1, subunit 2 (beta)	Hs.432970	NM_006431	6
BNO804	PTPRK	protein tyrosine phosphatase, receptor type, K	Hs.79005	NM_002844	6
BNO805	FLJ11918	cDNA FLJ11918	Hs.289068	AK095066	3
BNO806	TM4SF1	transmembrane 4 superfamily member 1	Hs.351316	NM_014220	6
BNO807	CHSY1	carbohydrate (chondroitin) synthase 1	Hs.110488	NM_014918	24
BNO808	TERF2IP	telomeric repeat binding factor 2, interacting protein	Hs.274428	NM_018975	6
BNO809	RDC1	G protein-coupled receptor	Hs.23016	BC036661	3
BNO810	BNO810	cDNA DKFZp564J0323	Hs.99766	AK095453	0.5, 6
BNO811	UBE2D1	ubiquitin-conjugating enzyme E2D 1 (UBC4/5 homolog, yeast)	Hs.129683	NM_003338	6
BNO812	KIAA0372	KIAA0372 gene product	Hs.170098	NM_014639	6
BNO813	CUL4B	cullin 4B	Hs.155976	NM_003588	24
BNO814	LCHN	LCHN protein	Hs.233044	AB032973	3
BNO815	PELO	pelota homolog (Drosophila)	Hs.5798	NM_015946	3
BNO816	BNO816	Unknown (protein for IMAGE:4052238)	Hs.348514	BC014384	6
BNO817	MRPS10	mitochondrial ribosomal protein S10	Hs.380887	NM_018141	6
BNO818	MGC10067	hypothetical protein MGC10067	Hs.42251	NM_145049	3
BNO819	KIAA1191	KIAA1191 protein	Hs.8594	NM_020444	24
BNO820	EIF3S2	eukaryotic translation initiation factor 3, subunit 2 beta, 36kDa	Hs.192023	NM_003757	3
BNO821	BNO821	ESTs, Weakly similar to L1 repeat, Tf subfamily, member 18	Hs.87606	BF131986	24
BNO822	UBQLN1	ubiquilin 1	Hs.9589	NM_013438	3
BNO823	PSMB3	proteasome (prosome, macropain) subunit, beta type, 3	Hs.82793	NM_002795	0.5, 24
BNO824	FLJ21962	cDNA FLJ21962	Hs.7567	AK025615	3
BNO825	FBXO30	F-box protein 30	Hs.95667	NM_032145	3

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO826	UBE2J1	ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	Hs.184325	NM_016336	24
BNO827	CDK2AP1	CDK2-associated protein 1	Hs.433201	NM_004642	24
BNO828	CRY1	cryptochrome 1 (photolyase-like)	Hs.151573	NM_004075	3
BNO829	BNO829	cDNA FLJ13364	Hs.378059	AK023426	6
BNO830	HSPC051	ubiquinol-cytochrome c reductase complex (7.2 kD)	Hs.284292	NM_013387	6
BNO831	C8orf1	chromosome 8 open reading frame 1	Hs.40539	NM_004337	24
BNO832	GNG11	guanine nucleotide binding protein (G protein), gamma 11	Hs.83381	NM_004126	0.5, 24
BNO833	PRO2013	hypothetical protein PRO2013	Hs.238205	NM_021243	24
BNO834	ZNF198	zinc finger protein 198	Hs.109526	NM_003453	6
BNO835	RAB11A	RAB11A, member RAS oncogene family	Hs.75618	NM_004663	6
BNO836	SMAP1	stromal membrane-associated protein	Hs.373517	NM_021940	6
BNO837	IGLJ3	immunoglobulin lambda joining 3	Hs.102950	NM_016128	3
BNO838	BNO838	ESTs, Weakly similar to hypothetical protein FLJ20378	Hs.319095	BU940787	3
BNO839	MTHFD2	methylene tetrahydrofolate dehydrogenase (NAD+ dependent)	Hs.154672	NM_006636	3
BNO840	PODXL	podocalyxin-like	Hs.16426	NM_005397	6
BNO841	BNO841	ESTs	Hs.406588	BM994036	3
BNO842	API5	apoptosis inhibitor 5	Hs.227913	NM_006595	3
BNO843	ERdj5	ER-resident protein ERdj5	Hs.1098	NM_018981	3
BNO844	HDGFRP3	likely ortholog of mouse hepatoma-derived growth factor, RP3	Hs.127842	NM_016073	6
BNO845	FLJ23728	cDNA FLJ23728	Hs.191094	AK074308	6
BNO846	CXCR4	chemokine (C-X-C motif) receptor 4	Hs.89414	NM_003467	24
BNO847	TUCAN	tumor up-regulated CARD-containing antagonist of caspase nine	Hs.10031	NM_014959	6
BNO848	MGC11034	hypothetical protein MGC11034	Hs.103378	NM_031453	24
BNO849	BNO849	cDNA DKFZp434G0972	Hs.106148	AL133577	24
BNO850	PCDH17	protocadherin 17	Hs.106511	NM_014459	24
BNO851	GALNT10	N-acetylgalactosaminyltransferase 10	Hs.107260	NM_017540	24
BNO852	CGI-111	CGI-111 protein	Hs.11085	NM_016048	6
BNO853	UQCRC1	ubiquinol-cytochrome c reductase core protein I	Hs.119251	NM_003365	6

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO854	RPL3	ribosomal protein L3	Hs.119598	NM_000967	24
BNO855	CMT2	gene predicted from cDNA with a complete coding sequence	Hs.124	NM_014628	24
BNO856	LOC116068	hypothetical protein LOC116068	Hs.136235	AL832721	24
BNO857	C12orf2	chromosome 12 open reading frame 2	Hs.140821	NM_007211	6
BNO858	PSMD7	proteasome 26S subunit, non-ATPase, 7	Hs.155543	NM_002811	6
BNO859	CCT5	chaperonin containing TCP1, subunit 5 (epsilon)	Hs.1600	NM_012073	3
BNO860	SEC5	homolog of yeast Sec5	Hs.16580	NM_018303	6
BNO861	SKP1A	S-phase kinase-associated protein 1A (p19A)	Hs.171626	NM_006930	24
BNO862	BNO862	DKFZP434C212 protein	Hs.172069	AK023841	
BNO863	CAPZA1	capping protein (actin filament) muscle Z-line, alpha 1	Hs.184270	NM_006135	24
BNO864	YES1	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1	Hs.194148	NM_005433	24
BNO865	DAAM1	dishevelled associated activator of morphogenesis 1	Hs.197751	NM_014992	6
BNO866	BAZF	Homo sapiens mRNA for BAZF, complete cds.	Hs.200272	AB076580	6
BNO867	FLJ13027	cDNA FLJ13027	Hs.200360	AK023089	3
BNO868	BNO868	DKFZP566C134 protein	Hs.20237	AB040922	3
BNO869	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1	Hs.205353	NM_001776	0.5
BNO870	LOC57228	hypothetical protein from clone 643	Hs.206501	NM_020467	24
BNO871	KIAA1463	KIAA1463 protein	Hs.21104	AB040896	6
BNO872	AF5Q31	ALL1 fused gene from 5q31	Hs.231967	NM_014423	6
BNO873	KIAA1376	KIAA1376 protein	Hs.24684	AB037797	0.5, 24
BNO874	ALDH9A1	aldehyde dehydrogenase 9 family, member A1	Hs.2533	NM_000696	24
BNO875	CDC42EP3	CDC42 effector protein (Rho GTPase binding) 3	Hs.260024	NM_006449	0.5, 24
BNO876	FLJ10326	hypothetical protein FLJ10326	Hs.262823	NM_018060	24
BNO877	MIS12	homolog of yeast Mis12	Hs.267194	NM_024039	6
BNO878	BNO878	hypothetical protein DKFZp761L1417	Hs.270753	NM_152913	6
BNO879	ATP6V1D	ATPase, H+ transporting, lysosomal 34kDa, V1 subunit D	Hs.272630	NM_015994	6
BNO880	VCIP135	valosin-containing protein (p97)/p47 complex-interacting protein p135	Hs.287727	NM_025054	6
BNO881	MGC11349	hypothetical protein MGC11349	Hs.288697	NM_025112	6

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO882	D10S170	DNA segment on chromosome 10 (unique) 170	Hs.288862	NM_005436	6
BNO883	FLJ39541	similar to RIKEN cDNA 9130404H11 gene	Hs.293332	BC042558	6
BNO884	ARPC3	actin related protein 2/3 complex, subunit 3, 21kDa	Hs.293750	NM_005719	24
BNO885	RPS19	ribosomal protein S19	Hs.298262	NM_001022	6
BNO886	BNO886	ESTs	Hs.30258	BQ228015	6
BNO887	KIAA0648	KIAA0648 protein	Hs.31921	NM_015200	24
BNO888	NEUGRIN	mesenchymal stem cell protein DSC92	Hs.323467	NM_016645	6
BNO889	CALD1	caldesmon 1	Hs.325474	NM_033138	0.5
BNO890	KIAA1160	KIAA1160 protein	Hs.33122	NM_020701	3
BNO891	NFIB	nuclear factor I/B	Hs.33287	NM_005596	0.5
BNO892	C20orf108	chromosome 20 open reading frame 108	Hs.352413	NM_080821	3
BNO893	HSPCA	heat shock 90kDa protein 1, alpha	Hs.356531	NM_005348	6
BNO894	KIAA0205	KIAA0205 gene product	Hs.3610	NM_014873	6
BNO895	C20orf112	chromosome 20 open reading frame 112	Hs.372610	NM_080616	0.5
BNO896	NSAP1	NS1-associated protein 1	Hs.373499	NM_006372	6
BNO897	SYT11	synaptotagmin XI	Hs.380439	NM_152280	6
BNO898	BNO898	clone IMAGE:5243590	Hs.397546	BC036880	6
BNO899	HNRPC	heterogeneous nuclear ribonucleoprotein C (C1/C2)	Hs.406125	NM_006321	24
BNO900	STMN1	stathmin 1/oncoprotein 18	Hs.406269	NM_005563	6
BNO901	ATP5B	ATP synthase, H+ transporting, mitochondrial F1 complex, beta	Hs.406510	NM_001686	0.5, 24
BNO902	PSMB1	proteasome (prosome, macropain) subunit, beta type, 1	Hs.407981	NM_002793	0.5, 24
BNO903	DDX10	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 10 (RNA helicase)	Hs.41706	NM_004398	6
BNO904	RPL36AL	ribosomal protein L36a-like	Hs.419465	NM_001001	24
BNO905	KIAA1462	KIAA1462 protein	Hs.46901	AB040895	3
BNO906	KIAA1199	KIAA1199 protein	Hs.50081	AB033025	6
BNO907	NDUFV2	NADH dehydrogenase (ubiquinone) flavoprotein 2, 24kDa	Hs.51299	NM_021074	0.5, 24
BNO908	C15orf12	chromosome 15 open reading frame 12	Hs.6118	NM_018285	
BNO909	DCK	deoxycytidine kinase	Hs.709	NM_000788	24

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO910	BNO910	cDNA DKFZp564F053	Hs.71968	AL049265	6
BNO911	MDH1	malate dehydrogenase 1, NAD (soluble)	Hs.75375	NM_005917	24
BNO912	SERP1	stress-associated endoplasmic reticulum protein 1	Hs.76698	NM_014445	0.5
BNO913	RPS3A	ribosomal protein S3A	Hs.77039	NM_001006	0.5
BNO914	ARHA	ras homolog gene family, member A	Hs.77273	NM_001664	0.5
BNO915	LAMA4	laminin, alpha 4	Hs.78672	NM_002290	6
BNO916	SNX9	sorting nexin 9	Hs.7905	NM_016224	6
BNO917	BNO917	hypothetical protein dJ465N24.2.1	Hs.8084	NM_020317	24
BNO918	RAD21	RAD21 homolog (S. pombe)	Hs.81848	NM_006265	0.5, 24
BNO919	SERPINE1	serine (or cysteine) proteinase inhibitor, clade E, member 1	Hs.82085	NM_000602	3
BNO920	PHLDA1	pleckstrin homology-like domain, family A, member 1	Hs.82101	NM_007350	6
BNO921	ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta	Hs.83656	NM_001175	24
BNO922	ELP2	elongator protein 2	Hs.8739	NM_018255	6
BNO923	THBS1	thrombospondin 1	Hs.87409	NM_003246	0.5
BNO924	ATP6V1G1	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G isoform 1	Hs.90336	NM_004888	24
BNO925	DNAJA1	DnaJ (Hsp40) homolog, subfamily A, member 1	Hs.94	NM_001539	3
BNO926	KIAA1238	KIAA1238 protein	Hs.236463	AB033064	
BNO927	CYB561	cytochrome b-561		NM_001915	24
BNO928	BNO928	EST		Figure 15	3
BNO929	BNO929	EST		Figure 16	6
BNO930	BNO930	EST		Figure 17	6
BNO931	BNO931	EST		Figure 18	6
BNO932	BNO932	EST		Figure 19	3
BNO933	BNO933	EST		Figure 20	6
BNO934	BNO934	EST		Figure 21	6
BNO935	BNO935	EST		Figure 22	6
BNO936	BNO936	EST		Figure 23	6
BNO937	BNO937	EST		Figure 24	6

TABLE 1 (Continued)

Novel Genes Involved in Angiogenesis

BNO Number	Symbol	Gene Description	UniGene Number	GenBank Accession	Peak Expression (h)
BNO938	BNO938	EST		Figure 25	0.5
BNO939	BNO939	EST		Figure 26	6
BNO940	BNO940	EST		Figure 27	6
BNO941	BNO941	EST		Figure 28	3
BNO942	BNO942	EST		Figure 29	6
BNO943	BNO943	EST		Figure 30	6
BNO944	BNO944	EST		Figure 31	6
BNO945	BNO945	EST		Figure 32	6
BNO946	BNO946	EST		Figure 33	6
BNO947	BNO947	EST		Figure 34	3
BNO948	BNO948	EST		Figure 35	6
BNO949	BNO949	EST		Figure 36	3
BNO950	BNO950	EST		Figure 37	24
BNO951	BNO951	EST		Figure 38	24
BNO952	BNO952	EST		Figure 39	3
BNO953	BNO953	EST		Figure 40	24
BNO954	BNO954	EST		Figure 41	24
BNO955	BNO955	EST		Figure 42	24
BNO956	BNO956	EST		Figure 43	6
BNO957	BNO957	EST		Figure 44	24
BNO958	ATP6	ATP synthase F0 subunit 6 - mitochondrial gene		NC_001807	24
BNO969	ND4L	NADH dehydrogenase subunit 4L - mitochondrial gene		NC_001807	6
BNO960	COX2	cytochrome C oxidase subunit II - mitochondrial gene		NC_001807	0.5, 24

References

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Dated this 28th day of March 2003

BIONOMICS LIMITED

By their Patent Attorneys

GRIFFITH HACK

Fellows Institute of Patent and

Trade Mark Attorneys of Australia

Figure 1

CNTATTTTATNNTTTCCTTTTTAAAAATTAAACTAAGTGGTTTATTACTGGAAATGAGATAATTTTCTTTACTAT
GCAAAAAGAATTTTAAAAGCCCTTTATTTCAAAATGTTTTCCATGCTTTGGCCCCCAATATCTTCAATATTAAAA
TAAAAATGATATATTTCATCACAAAAAGAACTGTGAAAAATAAAAATGTCTTAAAATCTGAAATACCTTTTGAAA
TATTACTGCCTAGTCACTCAAAAATTTATGTAACTTACAATTCTGGTTTAATTTCTTAATATATTATACATCTAC
CAAGACAATTTTCCCAAATACTTAAATTTTAAGAGGGCAATCTTCTTGACATGCAATAGTGTAAAAGCATGTTTT
ATTGTA

Figure 2

AGTACCAATTATTTGCACACTTTGGAAATGTGAATGTTTCAGCAATGTTTCATAGCTTGAAAAACACCGTATAAT
TTGGTCATCTAACACCTAGTTATTGATTCAAGAGAAATGTTATTCTGTTGGCACTGAGAAGGCTGTTGAATGAGG
AAGGATTAGATACATATTTTATGAAGGTCATCTTTAGAACAATACATAGGCTGAGTTAAGATACTGGATTTGAAT
TATTGAACATTACTGGGTATCACAAAATAAAAGTAATGCTTCTGGGGGACTTTACTAAAAATACAAATTCTCATT
TATATTTGTATTTGTATTGTAGTTCAAGGTTGGAACTCAGAAGTGTATATTTTAACATTGGTGGCCTTTGATAAC
ATTGTAAAGGAAAGACAAGTTAAAGTGAACCAACAAATAAAGAGACTGTGGAAAGTATCCCTATGGAATGTAAAA
AGGATCTTAAGCGGGACTAATTGTGGAAATGTAAGGATCGAGCTTTGAAATTATTGCAAAGGCATAAGTAGAATA
AAATGCTGACTAATCCACAAAATAGGCGTTTACGAGGATAATTCCCAGGTTTGGACTCTGCACTGTTGAATACTA
CTCGAACTACTCAAATGGAAAATTCTGATAGGACGCAATTACTCAGCGTTAAGCGCGGCCAACCAAACCCTGGAG
TTCACCCTGGCCTTTCCTGAGATTTAAGGGCGTTTTCGCTGAAACACCTACGTAGCAACTGGGCTCGGGAGATGG
AACGAAAAGGGCCTTTTCAAATCTTGTCGCCCCAAAAGAAACTTGAAACTACTCGCAGAACGGAGGCCCTCCCCC
ATCATAAAA

Figure 3

GGCAGGTACCCTCATTGAAATAGAGGATAGAGATTATAAATTATTTTTTTCTGTCTTCTTTTTACCTAGATAAGA
ATTTACCACATTTAGTCTGGTAAATAACCATCTGTATGGTCACAGAGAGCCACACAGAAATGAAAAAAAAAAAAA
AAAAAAAAAAAA

Figure 4

GGCAGGTACTTGAAGTATATTCTCTTTTTGGATTGCTGTCTAATTTTAATCACAAAATACAGAATGTAAACTATC
AACACCATTTTGCCTACTATGCATTTTGCACTTTAATAGCTGCTACTTCAAAAAACAGAACTAAAGGCCCATTCT
TTGGAACCATTTCACATTTTACAACTAAGAAGCAATACCAAGAATAAACAAATTCATATAACTCATCAATATGTA
TTGACTATTTATCTTAATTTTTTTCTTTCTTAAATATCACGATTAAAAAAAACATTGGCAGCATTTATAAGAACT
AAGTAGTTATAAGTAGTTCACTAAGAATGACCTCATCACAGGTTTTAGCTGCAACAATATAAAACTAAAGAGGTC
AAATTTATTCTCCAGGCTGCGACTACTTTTTCTTTTCTTTTTCCTTGAAGCATAGCTTACTTTGATTTTAACCCT
TCCTCACCACCCCTCTTCCTCTAAGTGTAATCAAGCAAGGAAAATTCCACT

Figure 5

TACTTTTATATCTATAGGAATAAACGAAATAAGAATTAAATTAAGATTTCATTAATAGTATCCTAGAAAAAAAAA
TTAGAAATGAACTTAACGAAATAACTGCACATACTATACACTGAAAATTATTTAAAATGCTGAAAGAAATCAAAG
AGCTGAATAAACTGAGATATACTCAATATTCGAATATAGCAATACTGAATATGAAGATCAAAATACCCAACAGAA
TTTATCTACAGATTCAATGCAAATTCCAATGTAGCTTTTCTTTAATATAAATTGACAAGCTCTTCCTAAAATCCA
TATAAACATATAAAGAAAATCAAACACACAACAATTCTGAAAAATAACAAAATTCAGTAACTTACACTCCTGATA
TAAATTCTTACTACAATGTTACAATAATTAAACAATATAGGA

Figure 6

CAGGTACCATCTCTCCAGCATGTTCCAGCCTTTATCAGTCAGTCCAGGCTTCTATAACAAAATATCATGGACTGA
ATTGCTTACACAACAGACATTTCTCACAATTGTAGAAGCTGGAAAGTGCAAGATTAAGGTGCTAGCAAATTTGTT
TCCTGGTGAGGGTCCTCTTCCGGGCTTGCTGTATCCTCACATAGCAGATAGAGAGAGAAAGAGAGAGAGAGAG
CACTCTATAGACTCTCTTCCTGTTCTTATAAGGACACTAATTCCGTTGATGAGGGCCCTATGCTCATGACTTTGT
CTAAACCTAATTAATTCTCAAAGGCCTCACTTCCCAATACTGTCACATTGTGGGGTAGGGCTTTAACATATGAAA
TTTGAGGGAAAAGGAATGTTCAGTCTAGAACAAAGCCCCATACATTTCAAAAAGCTGTGGGACAGGAGGGAATGG
GGATGGTATT

Figure 7

TACTTTTTTTTTTTTTTTTTTTTTTTTCCTTTAATAAGCATCGTGTTTATATATAAATGGCTTACATTTTTCCATG
TCCATATATGAGTCACACATGATGAAATGCTTGATGACTTACTCCTTTTTAAACTAGGTGCACTGTGGGACACCT
TTTATCTCAGTGCCTAAATTACCATTGCCATATAATAACAGCACTCAAATTAAGAACCGTTTCCACTAAAATTCT
ATTTTTAAGAAGCAATATTCATTTGTTGCTCTACTATGCTTCTTTTTCCATGCAGTA

Figure 8

TTTTAAATTTTTGAGGTTATAGAGTTTCCATAAAAAGTCATCAGTGATAATAAATTTTATTTGACTTTCTACTTA
TTCCGGATTCTGCACGAGCTGACATATAAACACGTTACTTATGGATCACTTTGAGAGAGGAAACACTATTCAGAA
GATTTAAATCCCAGAATTTAATCCATTGCTTTATTGCAAGTTTACAGAAGACTTGTCTTCAGTTTTATCTGCAGT
AGTTGATCTATTGACTTTAAATGTTGAAAACTTATACTAATGTTGCTTACTGTCCAAAATTATTCCTGGGAGATA
AACATTCAGATTTTCTACAATAACCACTGACTGGCCCCTTAGCAGCCATTTTAAATGTAAATTTGATGATATTGC
CCAAACATCCCCAGTCCAGTTCAACTTTCAGATCCTCAAGCCTACCATAGTCCTTTGTGTTTGATATTTCTTTCT
TATCCTTAAATGTGGTAGCTCCATGAGCACAGGGACCTGTGTTTTGATTCACTGTCTGCATACTTCACTTTGCAG
AACATTTTTAAGGTCCATGCATGTTGTAGCATGTATCAGAACTAGACTCCTTTTATGGCTAAATATTCCATTGCC
TGCGTATAGCATATTTATCCATTCAGTTTTCAATGGACACTTGGGGYGTTTCCACCTTTTGGCTCTTACGAATAA
TGCTGCAATAAATATTGTCATRTAAGTATCTGTTCAAGTTCAAAAAAAAAAAAAAAAAAAAAAA

Figure 9

CGGCCGCCCGGGCAGGTACTTCTTTTTTTTTTTTTTTTTTTTTTTACTTAAAAGTATTTTTTTATTTAATTTCTG
AGGTAAAAGACTTTTTTGTTTCAACTTCCATAACAAAATACAGAGCTATCAAATACCCCTGGAAAAAATTTGTAT
ATTATACATATATTTCTATAACATTACATCATATATACATAATATATATGCAAAAATTTGAAGACTTTATAGAAA
GCGGAACATCTAAAAGGCACTGCACAATGGAGTTAAGATTACTTACATTTTATGTACCT

Figure 10

TACAGAGTCTCTCTCATCACTTTCATAGCAGGACCCTACTTACCGATAATTCATAGCATACCTCCCCTTATTTTA
AAACTCTATGATAGCTGATTTCCTAGCTGTAGCAATCAGGATTCTTAGAAAGAATCGAAACTGAATTTAGCTAAC
TAAGGAAGCGGATTTCATTAAAAATATTGGATTAGTTTACAGAATCAGTAGTGGAGAACCAGGATTGCATAAAAG
GTAAGAACCAAGGGAGACTGGTCAAAAGAAATAATTTGCCCAGAAAGTCACCACCAACAATTCTAATAATGGGCA
CACGATGCTGACAATATTGCTAGATTCCAAAGTCTGTGGAGTTTAATCAGGCAAGAGCTAATGGCTGGCCAAGCC
TTAGTCATTAGTCACATGCTCCCTCAGTAGCATCTACGATCTTTGGATTCAGTAGCAGAAAATACATACCGCGTT
TTAAGTTCCATACCTTTCTTTTGTCCCCCAAAGAAAGCAGGTTTATACGCTGAGTCACCAAAAACAAAACAAAAA
ACACCCAGCAATATTCAAATATTCAATAATGTCATCAAAAATTTCATGATACCCAAAGGTTTTGTGTGTGCATTA
GCAATGTA

Figure 11

GGAGCTCCCCGCGGTGGCGGCCGCCCGGGCAGGTACGCGGGGGCCCTACATAAAAAAGATTATTAAGGCTAGGTG
TAGTGGCTCAGGTCTGTAATTTTAGCACTTGGGGAGCCAAGGCGAGAAGATCACCTGAGCTCAGGAGTTCCAGAC
ATACTTGGGCAACATAGTGAGACCTTGTCTCTACAAAAAAATTTAAAAAAAAAAAAAATCTGGGCATGGTGGCATA
TGCCTGTAAGTCCTAGCTACTCAGGAGGCTGAGGTGGGAGGATCACTTGAGCTCAGGGGGTTGAGGCTGCAGTGA
GCCATGGTTTGTGCCACTGCACTCCAGCCTGGGTGACAATGAGGTTCTGTCTCAAAAAAAAAAAAAAAAAAAAA
AAGTACCT

Figure 12

GCAGGTACTCTGTTTCTGAGGGTGGGTTTTTTGTTGTTCCTGTTCTTTTACTCTTTTATATACATACAGATTGGT
GAACCTAATTTTCTCACTTTTTCAGTTTATTTCTTTGGGACAGTTTTATGGCCTTAGAAATATACTAGTCATTAT
ATTTTTTTTCATTAAATTCCTTTATCCAGGAATTATGAATAAATCTGGCATTATCAAAAAGTTTAGTCACTTCAAC
TCCATGAATAGGAAACAAATGGTAACTTGACATCAGAAAAAAAAAATTGAACCCTGTTCATTTTCTCTATCTGAAT
TTTATTATATTTTATGCTACTTATTTTAAAAATATTACTTATTTTATTGTATTTGGTGAATTTTATTTTATTCTA
CTTCTCTGAAAAAATGGTCATCTATTCATAGTTCGTGTTATTCCTCCAGTTAACTAACTGATGCAGCAGTTGATG
GGAACTTCTCTGTAACACTAGATACATGAGATATTATAATATTTTTTCTTTTTCTTTAGTTGTTGTATAGTCGTC
TATCTCAGCTAGTAGAATTTAAAGTGAAAGGCATAAAAATCTGAGGTGCAGATATGAAACACAGCAGTTGTTTTA
TATATAAATATCTGGTCACTTCTGTAAGCAATTTTTTTTTTACTTACTCAGA

Figure 13

GATGACCATAATCATAACTTTTGGAAACTGGGGGATCTTTGCCCACCTTCCCAATAAATCTGGGAGTATACGCCA
TTTTATAATCTAACTATGTCTTATTGTCCAAAATAACATTACCAAACCCTGACATTTAGTTTCAAAAGTAAATTC
AGCAATAAAATTGGATCCCAGACTTTAAGGAGACTATGTATGTCAGTGTTACATCTATTGCATCTAATATAGGAC
CCTAGCTGTGAAGGCCTTTAAGAATAGTATAATTTTATGGAAGCATGCCTTTTTTGGCTTGTAAAGACTACCCTC
TTGACTTTCAGAAACTAAGTCATCACCTTCACGTATAATTAAATCAAAGATGCAAATTCTCACCTAAAATTTACT
CATACATATTTTAGAAACCCATAGAACTTTAATTTCTTTTTTTCTATACGGCAACCCAAATCCTTATGAATGCGA
TAAGGTAATAAGAAAGATGTCTTAAAGAAAAAACAGAATAACTGTTACTTATGGGGGCCAGACAATCAGCTGTTT
TTTACTTAAGGGTA

Figure 14

CAGGTACTACTAAAATTTTGTGTTTGAGAGGAAAAAGAGAAAGGTAAAAAATAATTCNTGGGAATATTTAAATCA
GTAATTGAATACATGTAGTGATGTCTTTAAAGCACCTTCAAGAAAGACCTCTTTCATTTTGGATGACAATACATT
AGGATAAATGAAATATGTGAATATGGTAATTTTCCATAAAAACAAAGGAGACTATGGAGACTGTCTGGTTCCTGA
TTTGGCCATACAAAATATAGAAAATTCAAGTGACCCAGGTTCCACAGTCCCAATTCTGTCAAATTCCGATCCAAC
AATTTGTAAAATGGATAACATCTGTATACTTCCACTGTATATGAAAAAGAATGAGCAGCTCATAGGGCTACTTAA
AAAAAAAATGGAAAAAAAA

Figure 15

GTTTANGGGGATNNGTTTCTAGAATGGGGCTAGTTGTATCTAGGCAAATGGGTAACTCTTAATTATTGTATGACA
TTTTTTTCTGTATTTAATGGTTAAGATTTAGAGGAAGGGGCAATATGTGAAAAAAACACGTTATGAGAATGTTTC
ACATTTAGACCTTGAGAAATTGGTATTTATTTATGCCAGTTACCTATGTCTCTGAGGTGCCAAGAGGATGAATTA
ATATTTTATTTCACTTGTGGAGATATTCAGAAACATTAAAGTCCTTGGTCCTCATGAGAAAAGAATTTTCTTGAG
ACTTGAAAGCATTACTGCAGACATTTTGCTAACACATTTTTTGTGAGCTGTGTTAATTGCACCTACCTTTATCAG
TCAGAAGTTTTGCTGATTTAAATCAGATCCCAGCTAACCCAAGCGGGGCTGCACTGAACGCCACATCTCTATTGC
CCAGATTTCCCAGGGGACTCTGGAAATTAACCAGAAGCCCTCCTGCTCGAAGACCAACTCTTGAGGCGTATATGA
TTTGCATAGAGGTTCCAATGTGCCAAGTTCTTAGAAGTTTCTGGGAGCCATACAGATGATTACTATTCCAAATGT
TCTCATCTTGGGTTCCACCCTTTTTATTCAATGAGATTTGACTTTTCTACTTTTTCACTCCCATCTTCACCACAC
CAAAATTCATATCCAACAGTCATTGTGTATATTATGAAAGTTGGTCTCAATTTAGTCAAACCTGGGTTTCAGGGG
ATATAGCTCACATAAAAAGGAATGGGAGGTATCATTATATGGAAATACCTGCTGTGTGCCAGAGCATCCTTTAAG
GATTTTGTTTTCTGTTGATATTTTTCAAACCCTCCACTTTACAGAAGAGGAAAGTCAGGCCCAGAGAGGTCAAGA
AAATCACTTTCAGGCAAACAAGGTCACATGGAAAATAGGGATTCCTGTGAACTCACCCTGTGTTCTTCTACTCCT
TCATGCTCAAAAACTCTCCCGGAAATCAAATGAAGCCCCTGCTGAAGGGGGGCCTGGTTAAAGGGGATTCACAAA
AAGCCAAACCCTATTTGTC

Figure 16

ATATTTAAAAAGTAAAACAAACTATTTGGAAAAAAATGACAGTAAAAACAATAGGAAAGCTTGTAAACTTCGGGC
TTACTATCAAAATCACTACCTTAAAGTAATTTACTACTTTTAAAATAAAGAATAAAATTAAATAATTCGGCATTT
AAATAATAAGGTTAGAAAGCTGCAACTATTCTATATGGAAAAGGATAGAAAAGAAAAAGAAAAACAATGAATACA
ATTAGTAAATCCAAAAGGAGGATGATGATAACTGTTGCAACCTGGGGAGTGGGAAAAATGTGACTCTTTATAACA
AAAACTCTACTTTTGTGCCTGATGAACACTTCATAAACAAATTTAAAATATATAGATAAATCCAATGATCAGTCC
CTTAAAAGGGCAAATGTAAAGTA

Figure 17

CGCGGTGGCGGCCGAGGTACTTTTTTTTTTTTTTTTTTTTTTTTTTGGTGTCTGACAGAATTACTAGCTATGCCAT
TAAAGCCCAGATTTTCTTTATTGGAAGTTTTAAACTACTAATTCAATTTATTTACTCTATATAGGTCTATTTAGA
TTTTCTACTTCTTTTGGAGTCAGTTTTGGTAATTAGTATCTTTCAAAGAATTTGTTCATTTCATCTAAATTGCCA
AATTGGTTGTTGTAAACTTGTTCATAATATTCCTTTATCTTTTTAGTATTTATAGTATCTGTAGTAATACCCATT
CTTTCATTCTCAGTATTTCTAATTTACATCCCTTCTTGTTCATTTTAGCTAAAGATTTACTAAACTCTTTAAAGA
ACCAGCTTTTGATTTCCTTGATTTCTGTTCTGATCTTTATTACTCCCTTTCTTCTACTTACTTTGGTA

Figure 18

CGGGCAGGTACTATTATATAGTTGCAAAAACTAAAAAAAAAAAAAAAAAAAAAAA

Figure 19

GGCGGCCCCCGGGGCGGTACCACGCTCCCGACTGTTCGAGGTACGCTCTGCTCTCTCAGTAGCCAAACAGATAAC
AGCCAGTACGTTGTTATCCGTATTTTCGTTCGTTGTTTTAACCTCAGTAGCACATTTGGACCTTGGGTGTTATTA
TATCTTTGTCTTTATAATAAACTCATGCACTTGCTTCTAAGATTGATACTTGGAGCTTGCAACTTATTCACTCAT
AAATGTAACTTACATATGCTTTCTAGATTTTGCACTTCTTCCCCTGCACCTTGGTAGACCTACATTGAATTAATT
AATTTAATTAGTTCAAACATTTATTGAAGAAGAACTATGCAGTAGGCTCCCAGGATCAAGCGATGACTTAGTCTA
CCTTCAAAAG

Figure 20

GGTACTTTTTTTTTTTTTTTTTTTTTTTCTATAAAATCATCATCTTCACCACCTTATCAGGGGGCAACTTTTATTT
AATAACACTTGAAGTGTTTTTACTTGGTCAATTATGGATAAATAATCCGGTTTGCCTTTCTACGCTTACTTAAAG
GAGGCTGTGAATTGTTGGAAAAAGTCCAAAACTGCAATTATTTGATCCACTATTAACTCCTTAGTCTCTCTTGTT
GACTTCCATATCAACATGTAACATATGGGGTATTCTAAGCCCTTTGCACGCCCAATTCAGGAAATCCAACTGCTG
ATAGGACTCCCCCTCCTCCCTATCCCATTTCCCCCTACTCTTAGCAAAACTGCCTCTTTCCCAAACTCATCTCCT
ATCCACCTCAAAAATGTTGTCCTTTATTTTCCTTTCCTCCTATCTCAAAAAAGTGTTTCTCCGAATTTTCAAGAT
TAGTTCCTATATCTGTGCTACTGATAGCCCCTCTTGCTTTATCAGTTATTCTGTTTTTCCTCTTCAGTCTCTCAT
TCTACCCTTCTTCTTTCACAAGTGCTCAAAAGTAAACAGATGAACAGACAAATCGGTCGCTAAGTTTTACTTACT
TGCCTGCCACAAACTCTCATGCACTTATTCTTCTGCATTTCAGACTCTACCAATTTAACTTAGGCTCTCATTATC
CCTCCCTCAAAAAGTAACCAAACATTTCTGTATAGCCAATCTAATCCTCCAACAAACATCTTAATCATAATACTC
ATGTATTCAAAATTCATCCACAAGCCCATCATAGAATTCAAACACTTTTTTTTCTGGCTTTCAAATTTTTCAACA
ATCCGGCTCTGATCAACCTATCTCATAAAATGATACTGCATTGCTATCTATTATCTTTTATCTACCTACAAAATT
CAATAAAATATAATCTACAAATTTCAGTAAAATACAATCCAAAAAATCCCTCCCTTTTCCCTACTACCTTAATTT
CTCCGTCCTTTATTGAAAAAAGTGCCCTTTAAACTGAAA

Figure 21

ACATAGAAGGGAAAAATGGCTTGCTTATTTCAAATAGTTTTGAGGATGAAGCACTATGATGGAGATCCACTGAGG
TAAAAAGGATCCTCAGCTTTTTTGATTCAGAAATGAAATATTGATGATTTCAATCAAGATTGATCAAAACCTGAT
GAAAATATCCTGATAATCAATGGGAAGTAGAATTACAGCTTATTGTGAGAGTCAGGG

Figure 22

TTTTTTTTTTTTTTTTTTTAAAGTTGGAGCTACTCTGCCCATGAGGTAGCCACCCTTTTATTCCTTTTCTTAAAA
CAAGTTGGAAAACCATTGCTATAGATAAACATTCGATTTCATTCTTTTAGAAATAATACATACCTATATATTAAT
ATATTCAATATATGTATCAGTCATATATATATTCATTAAAATGTTAACACCCAAGTATTTAGATTAAATGCTGTT
ATTTGGCTTATAAATTAACAGTA

Figure 23

CGCCCGGGCAGGTACAGATATGTAAATATTCCAAAATCCAGAAAAAAAATCCAAAATCTGATATATTTCTGGTCC
CGGGCATTTCAGATAAGGGATACCCAACATGTGTTTGTAACTTTCAATACTAATGAAATTAGTAAATTTTGTTTT
TTACATTAGGTGCCTAAAACTCTTGATTTTACACCAAAAAGTAATAGAACAAAAATAAAAAGCTATTATATGGAA
TGGCATCAGAGTCACTCTGGAGCAACAGGAAGCTAACTCTGTATATCAACCATAATAGCCTTATTACTCCCAGAA
GGACATAGTTAGAAGCATTTCTGGTTACTCTTCATATTAAAATCTTTGGTGTTTTGGCTTCAATACACTCCCTTA
ATGGGTGTTATTATTCCATTGTAATGAAATAATATTCCATGGTAGCAGAAGGAATGCTTAAATTCTGCCTTACTG
TTAAATTCTATATAGATACTTGGGTTGGTCAAATTAATTTGGTGCTTCACCCAAAGCCCCAATGTATTTGAACTT
TAATCTTTCATAGGAATCTAGAAAAGCACACAATTTAATTACCACTACTATTCATTATCAAAGATTGCATGAATG
AGGTTTAAAACAATAGAAAGAAATAAAACTTTAGCCCCCTATTGCTAGATGTTCTGGCCCATGAGAGAGGCAGGG
CAACTTAAGAGCTTTGGAGGCAGACAGGGCAAGACTGTAAATCTTGGAGCAACAATTTATTGGCTATGTAGCCTT
GGAAAGGTATCATTATTTTTCTCTACCTCAGTTCACTAGGTGGAAAAATGGAATAACAGCATCTAACTCAACAAA
TGTTGATTATTACAAAAACTAACGATTCAAAAAGCTAATATAAATAAAGCATCATGTTATGCATCTTTCTCATAA
AAATAAATCTTAATGAAAAGCATCAACTTTTAGCTTTATCCATAAAAAATACCAGACAAAACAAAAAAAAAATTC
TAAAAATCAAAAAAACTTCCTAATCCTGAAAAAGTAAAATTAATTATGTTCTTTTGTTTTCAAAAAAAAAAATTA
TTAATTTTGGAAAAAACATTTTAAAAACCAAAATTTTCCTTTTTAACTTTTTCCCAAAAATTTTTTTTTTTTCCC
CCAAAAC

Figure 24

TACTTCTGTCTTCCAGTTTTCCACTTCAAACTTCTATCTTCCCCAAATTGTTTCATCCTACCACTCCCAATTAAT
CTTTCCATTTTCGTCTGCGTTTAGTAAATGCGTTAACTAGGCTTTAAATGACGCAATTCTCCCTGCGTCATGGAT
TTCAAGGTCTTTTAATCACCTTCGGTTTAATCTCTTTTTAAAAGATCGCCTTCAAATTATTTTAATCACCTACAA
CTTTTAAACTAAACTTTAAGCTGTTTAAGTCACCTTCATTTTAATCTAAAAGCATTGCCCTTCTATTGGTATTAA
TTCGGGGCTCTGTAGTCCTTTCTCTCAATTTTCTTTTAAATACATTTTTTACTCCATGAAGAAGCTTCATCTCAA
CCTCCGTCATGTTTTAGAAACCTTTTATCTTTTCCTTCCTCATGCTACTCTTCTAAGTCTTCATATTTTCTCTTA
AAATCTTAAGCTATTAAAATTACGTTAAAAACTTAACGCTAAGCAATATCTTAGTAACCTATTGACTATATTTTT
TAAGTAGTTGTATTAATCTCTATCTTTCAAAGAGAAAAAAACTTATCTGCGGTTTCCTCAAGCTCCGCCTGCCCC
CTCAGCAGAAGCCCCTGCGCTGGCTGCCTCAATGCCTCC

Figure 25

TCACTTAAATATAATAATGATGTTATTAATAAACGTTAGCTTGAGATCAAAGTGTCAACAAGGCATTCTTTTCTC
TTTTGGTGGTGGTAGATTTCACCCACATATTTTATGCATCTATAACAAGTCCCACATAGCTGGCTGATTACTGAG
CTTTGAGCAGGTAGCACTGGTCACTATGCACAGATTAGTATGATTACTGAGCTTTGAGCAGCTTGAACAGATCAC
TATGATATAGGCTCTGGAAAGGTTGTGCAGATATGCCTGATGCCAAGGCACTGGCAAAATTGTTGGTAGGCTGGC
AAGTTAGTTCCTTCTCAAGGAAAAAAAAAGTGTGAATGATCCTATAGGTTACTAGACAGACATGCAGTTTCCCTTT
GGAGAAGGTTGACTTTGGACTTTGAGAAATACATTTTTTTTCTTTTAATCTAAAGAGATTTGAAAATAATAGATG
TCCAATTTTTGTTTACCTATTCTGAACTATAAGGCACATTGCTTTGTTCTTCTGTGCCTCAGTTTACCCCTTTTA
GTAAAGGAGGTCTATTTTAATCAGTGAGAGCTTCAGGTAGCTGGAGGAATGCTTCAGGAAACACAAAACACAATG
AAAAAATTGTTGAATCTTAAGCTGCATGTTGTGGAAAAGAACTGTA

Figure 26

AGGTACTTTTTTTTTTTTTTTTTTTCTTTTTTTTTTTTTTTTTTTTTGTAACTATAATATTTATCATTTTAATT
GATGTGTATAGTTTTACAAAATCAAATAGTAATAGAAACAAAATAGCATCCCTTGTGCCTCCCCTCCCTATTGCT
CCCTGGTCTTATACAGTCATAAAAAGTCACAAGAAACCACGTACAAATTGTTAAGCCATTTCTTCTGCCATTTAC
CTTCCAATTCCTAAGGAGTATGCATGTGCTGTTATTTATTTTAAACATTATCTTTTTTTCTCCCTGTCTTGACAG
ATGTTATCTAACTCAAGTGCACTTTGCTCTCCCTCCCCCATTCCCCTGGTGTAAGCATACCACACAGTCTTTGAG
TTCACATTATTATTTGTGTCATATAGATACTGTTCACATGAG

Figure 27

CAGGTANCACTTGAAGTCAAAATGCAATCACAGTTCTTTTGATCTTTTTAATCAGTTTCTCCATTGGACATTTTT
CTTGCATTGCCTCAGTAACAGAATAGAGGAGATATATTTTGATGTTACTTTTCTCTTGTCACTTTTGAAGGGTGG
TGTTGAAGAGATGCCACCATCAGTTGGATATATAAAAGAAATAGCCAAATAAAGAGATAATCTGTCTCTCCCATC
AGCCCCTGAACAATTGATAGTGGAAAATAATGTAATACAATTTATAATGTTCAAGATAAAAGCTTATTGATAATT
TTTTATCAATAACCATCAAGGATGATGTAATTACAAAACTCAAGATGATTTGACAGAATTTTTCAAATTATTTAA
ATCTAAATATTCAGACTGAATAAACTTCAAATTAGTTAAGTGGATTCATTTGATAGATGCTTTATTTTACTCATT
AGCCAATAAAACTCTTAAGAGTTTTCTTAAGGTCTTAGGACAGACAGATTTATGCTTCAGTGACAAAAATCAAGA
ATTTAATCAGTTACACAAGGAGAAAGTATGTGTGTATGTGTGTGTATACACATAACCAGATATTCTCCTAAGTTT
TTCAAAATAATAAAAACAGATATTTTGGGATTCAT

Figure 28

GTACTTTTTGTAACATACCTGTGATGCTGCTCCAACATCCCTGCTAAGAGCTTTGCTGATTTTAACAAGTATGTG
ATTCCACTGCTGTAAGCCAAGGGTAAACTTGATTCCACCAAATTTGCTTAAGTGTTACTTCTCTGTATTAAAGCT
TCAAGTTCACTCACTGTCTAACCAATGATACACAGCTACCTGAAGACATTAAAATCTTTCATAAATTTATTCTCA
ATTTTTTTCTTCTTATAAATTACTAGCATATCAGGATAAAGAATAAAGTGTGTGAAGAGGCAAAAT

Figure 29

AGAACTCTANTTTGGAGGGTAACAAAATTTCAGGGACATATAAAAGAAGTTGCTTATTTATTAGACAAACAAAAG
TCACCCAGCAAATATGCCATGGCTATTAAAAATAGATTGTCCAGGGAGGAAGAATAAACCACTAATTAAACACTT
TTGTTTCTCTTTTTTTTAAGCCATTAATACTGATACCTAGAGAGAAAAATAAAAACCAGAAATACTTTCAAAAGC
CAAGCATGATAGACTAGCATTTATCAGTTACCTTTTTATTTTCAATCCTCAAGAAATGCAATCGCCCAAATAAAT
TTTAAGTATACCAAAACACTAAGAAATTACAAAACTCAATAGCAAAATAACTCACGTGTTACAACCATAATTACT

TCTATAACTAGAAAATTATTATCCTCATAGGAAATCCTGGCAAATTGCTGAAAACATTTCTCTTCTGAGTTAATC
TAGGAGGGAAAAAAAATAAAACTTCAGAGTCCAGTCACTTTGAAGTCCTTATGCCCAAAAAGACATTATCTCCAT
CAATTGTCTACATGCGAATAATTTCAAATGTCTTTGTCTGTGCAACAGCCACATTTCCTTCTCTTATGATGTTTT
TCTTCTCTCCCTATCCCATTTTCATTTTCACAGCTGTTTCTGGAGAATAAAAATGAAGGAACACTTTCTTGCTCT
GCCTCTCCATGCTCCACTTTTTACAAGTCTTTGTCTGATTTACTACAGAGAACTATAACCAAACCCAAAAAAAGA
ATGGCAAGCGAAAGTAGGAGAGAAGAGTGTGCTTTTGCGCTCAGAAGCCACCACTTCTCAGGTGGAAAATAAGAA
TGTCATGCCATGATGACATTCTGCCTTGTGTCACAATGATTAATAGTCATAATTTTTTCCCATTAATGGGAGTAA
ATGCAACCAAGTCGATGGAATCTGACCAATTTTGCACAGCATGGGTCAGTGGAGTGCTATTATTTTGGATGGAGA
AATGAGAACCAAAAAGGAATTATTTTTCCATTTAGCACCTAAAGTGGAAAAATTAATTTTTTCATCCCCCTTTTT
TTTCCAACCTAAAAAAAAAAAAAAGAAAATTTTTCAAAAAAAAAAAAAATTTTTTTTTCCAAAATTTTACCCCCTTT
TAAACCAAGTGTTTTCTTTTTTAAATTGGGAACCCCGGC

Figure 30

TACTGTCTTAGTCTGTTTGTGCTGCTATCACAAAATACCAGAAACTGGGTAATTTATAAATAACATAAATTTATT
TCTCATGGTTCTGGAGGCTGGAAAGTCCAAAATAAAGATGCTGGCAGGTTGAGTCTGGTGAGGGCCCAGACTTTG
CTTCCAAAATGGCATTGTGTTGCTACATCCTTCAGCAGGGACTAATGCTATGTCCTCACATGGCAGAAGGGCCAG
AAAGGGAGAAGGGCCTAGCCTTAGTTCCCTTGAATCCTTTTATAGGGGCACTAATCCATTCACAAGGGTTCCACC
CTCATGACCTAATCACCTCCCAAAGGCCCTACATCTTAATATCATCACATTGGCAAATAACTTTTAATATATGAA
TTTTGTGGGGATGCATTCAGACCATAGAAGATGCTCTTTGTTTCCCTTGAACATTACTGTTTTTTTAAGGATTTGG
CCTCAAATTCCTTCTCTTGATAGATCTCCTTTTGGAATTACCTATTCTGAAAATGCTCATGTTTCACTAATTTTC
CTATGACTTTTACATCTGTATCTCTCATCTTGAATTCCAGTTCTACATTTTCAAATGACTCATACGTGGACAATG
CACTGTCAGCAGAAACTCAACATTTGTAAGGCAGGACTCTTCAATTTTTCCTCATTTTGATCTCCTCCATACCCT
TTAATTGTCAAGGACATACTTTTTAGGTGTCCAGATTCAAAATTTTAGAAGCATCCTAAATACCCTCCTTTCCCT
AATCCCCACTTATCCCCTCAGTGACTTAATCTTGGAAATCATTATGATCATTTTCATAAATGTCCTTTTATTTTT
AAAATGATTTTGAAGATTTTTTTTCCATAAAAACCTTTTTTTTTTTCTGGC

Figure 31

CGGTGGCATTGCACTCGTTCGTTGTTAGATATGGAAAACCAATTGACTGTTGTATATTAAACTTGTATCGTGCAA
CCTTGCTATAAATTAGTTCCAGGATTTTTTTTTTTTTTTTTGGTTGGTTCCTCGTGGATTTAGCTATGTAGTCAC
ATGTATCGTCACTTGTGAAAGAAAAACACAATGTTATTACTTCCATCGCTCATATATCCAATCAGTAAACTTACC
ATAGTGAAAAGAATATAGCTACCACAGCTAGTCTCTCACATGACAGATAAGATGAATGGATAGCGCATCAATGGT
TGACAACTTCTCTAAAGTAAATACGCGCTGGCTGTCTCTTTTCAAGATTAACACAAGATATGTGTCAAACCTACA
AACAAGAGGACGCTTATTCCCTCGCCTCTACAGTTATTGAATACCTGGAGCTGCACGACTTCTATATCAAACTTA
CAGACCCCGTCTACCTCTAGGGAGGAGCACCGGACTCGCTCAAGACATCGTATGAAAGAGTCTCTATAACCCGCT
GCTCTATTCGTCGTCCCAGAGTGACCGTCCCAGCCCTAGCTACATCTGTGGAAATACCCGAGTTAATACCCCTTA
GCAGGTTATCCCCCGTTCGATCAACAAGTTGTGATAGCCCAAAAAAGCGCCCGACACAAAATCAACCTTAGCCTA
ACTATTAGAAACAAACGACCAACCGCCCAGGTGCACGGTAACCATGAACCCCACCGCTATCACCCTCCCGCTTTG
ACGCGGTGCACAACCGCCCCCCGCGCTCCACCAACTACCCCTTATTCTGGGAACCACCTCTCGCCCCGCTCCTCT
TTCCATTACCCCCATTACAATTGTCCCGGTTCCCTCCACGCCCTACTTATCCACCTACCAAAAGCCCCTAAACTT
CCGAAACGCCTCTCTTCCCACCAGTTCCACAAATATATTTCAATTTATCACCGGGACAACCACCCCCCCACAAAA
ATCTTTATTCACCGGCCTCTGGGATTTACCTGATATTGCGCTTCAACCCTCTCACCACCAGACACATTTTTATCA
TCCTATCGCGCAGGATTGCTATGCCCCCCCCTACCTACTTGAAAGAAATCGAATCTAAGGTTTGTCTACAACCTTA
CCCACCACTCCCCACTCCAAGCAGCCACATATCTTGTCCACATTTATTTTTTCTAATCCTCAGTAATGGAAACCG
CTTCCGCTCATATCACCCAACCTACACTGTCTCGGTTTGAGCGCTGTATTGGTTGTTGCTTAGACAATAC

Figure 32

GGGGAGCTCCCCGCGGTGGCGGCCGCCCGTGGCATGTACACTGTAAAGTGACAAAACATGACTGAAAGAAATTTT
TAAGAAGCTAATAAAAAAGGGCGGACAGCCCATCTTCATGGATTGGGAAGACTTAATACTGTTAAGATGGCAATA
CTTCTCACCTTAGTCTATAGATTCAATGCAACCCACATCCCACTTTCCTTTACTGCAGAAATTGACAAGCTGATC
CAAAAAATTGTAAGGAAATGGCAAGGGATCCATAATAGACAAAACAATTTTGAAAAAGAATAAAATCAAAGGGCT
TTGTAACACTAAGGTTATAAAACTCTTATAAGAAAATAAAGGAGGAAATCTTTGTGACCTTATCTCAGGCAAATA
GTTTCTTTGATATGACATCAAAAGCATACGTAATAAAAGAAAAAAATAGAGATATTGAACTTCATCAAAATTAAA
AACTTTTGCATTTCAACTATCAAAAAAAAAAAAAAAAAAAAAAAAAGTACCT

Figure 33

CCATAGATAGAAATATTAATACCCATGAAAGAGAGGACAATGAAAGGTTTGTATCATTTGTATGTCACAAGTCAA
CTTTTTTCAATCACTCATTATTAGTTTAACTGTAAAAAATTATTTACATTTAGCGTGAAACTTTCCTGTATTCTC
AACATATTTCCTTCGCGTAGAAAAGCAAACCTCCAGTTCTCTGTTCTTTGCTTGGATACTTGCCAGTTTGTAACT
CAGCTATCAAACAGTAAAGCTCACAAAACACTTATTAAAATGACTAAAATCCAAAACACCAAGAGCACAGCATGC
TGGTGAGATGTGGAGCAACAAGAACTTTCATTCATTCACTAATGCTGGCAATACAAAATGGTACCTGCCCGGG

Figure 34

CGGCCGAGGGTCAGGGCCAAAACTGGGAACCAAGGATTTAATAACTATTATGATCAAGGATATGGAAATTACAAT
AGTGCCTATGGTGGTGATCAAAACTATAGTGGCTATCGGCGGATATGATTATACTGGGTATAACTATGGGAACTA
TGGATATGGACAGGGATATGCAGACTACAGTGGCCAACAGAGCACTTATGGCAAGGCATCTCGAGGGGGTGGCAA
TCACCAAAACAATTACCAGCCATACTAAAGGAGAACATTGGAGAAAACAGGAGGAGATGTTAAAGTAACCCATCT
TGCAGGACGACATTGAAGATTGGTCTTCTGTTGATCTAAGATGATTATTTTGTAAAAGACTTTCTAGTGTACAAG
ACACCATTGTGTCCAACTGTATATAGCTGCCAATTAGTTTTCTTTGTTTTTACTTTGTCCTTTGCTATCTGTGTT
ATGACTCAATGTGGATTTGTTTATACACATTTTATTTGTATCATTTCATGTTAAACCTCAAATAAATGCTTCCTT
ATGTGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGTACCTGCCCGGG

Figure 35

GGGAAAGAAAGGGGGAGCGCCGGCCGGGGGGCGGCCGCCCGGGGGCAAGTACTCTAGAAGTGAGCATTAGCGTGT
GTGTTATTGTTCGTCGAATTCACATCGCAAGTTACAACATGTGCTCTATCAACACGATTTAAAGAAATATGGGGT
TGGAAATTAAAAATGTGCTATTTCAAAAAACAAATCTCAACGCCCATTCCTTGGGACCATTTCTCTTGTTAGAAC
GAATAAGCACGCACCAGAAAGAAACGAATTCATAAAACTGATCAATATGTATTGACTAAGTATCTTAATTTTTTT
ATTTCGTAATATCACGATTAAAAAAAACATTGAGAGCATTTATAAGAACTAAGATGTTATAAGTAATTCACCCGG
AATGACCGTCCTCACAGGTCGCAGCTGCAACACTCTATAACTAAGGAGGACAAATTTATTCGCCAGGCTGCAACT
ACTCGTGCGTTTCTTTATCCTTGAAGCATAGCTAACTTTGATTATAACCCTTCCTCACAACGCCTCTTCCTGTAA
GTGTACACAAACAAGGAAAATTCCACTTTGAAGTATCT

Figure 36

GCGCGCGGCGGGGAGGCACAGCCTGTTTAACTCGTTAATGCTGCATCAGTGATAGATATTTCGCGAAGCGGGAAT
ACACAATGTGTCCAGGGTGGTGCTCTTTTGTGTTGTTTGTATGGTTCAGAGGGGAGGGTGGCTATATCCTTGCGA
GCTAAGGAGATGCTCAGGCTTACACACTACCTGGTGTCCAGCGAATGACTCATCTTACAGCATCACGAATATGTT
GGCGTACACCAATACCTTATCCACCCGTTCTGACTGCCTTAAATGGGTATTACAGGAGAAGACTTTGATCCATCG
CATCCTGAACGTCATCATTGGTGAGAGGACAACCGTCCTTGTACTATGACCATCTTCTAAACAGACATGCATCGG
ACCAGAGGAAGATCGGCTGACATCGTGTATCTGCGTGCCTATGCGTTTCCGCTGTAGCTCCTTAGCCCTGTGGAC
ACAGTATTTGGACTGCCTGTTAAGTTACGTAGGCACTGCTTGACGGGTTCTCCCACACGAAGATCCTCACGTTGA
CACAGATTTCCTGTTCATCTTATGTGTCTGGTCAACTTGTTGCCCCGGCCCAACATGACCTATCCCTTCTACGGG
TTCACAATAGTACCGTTCCCTAACAGAATTCCTCACGAACTGTTACCAGTCTACAGGAAAAGCCATTACCCTGAC
TCTCTGACTTTGCCACACTCAAGATCCCCTGCTCTACGACAAGGGAAGCAGACGTCAGCACCTATAGTTTACACG
TTTGATTCTTTCTTGTTACTTTGACGGTCATACAGTGTTATGCGGAAAGTATCACAAACTAACCGAACGTGCCCC
AGCAGACATCCTCCGCAAATCGAAACCGCTCCCCATTCGAGTTGACATGTACACCAACCTCTCTTCCCTGTCTAT
GCCTATATTATGTCAGCAGAATTCTTTAAAAAAATTAGTCAGTTTGCCTCCGCTTTCGGTTGGACTCTCGCACCC
AAAGCGTACCGAACCCTTAACCCTCCAGATGCCCCCGCGTGTCTCCACTTGTCTCCAATCCTGAGGGCTCCGCCC
CCTACCCTTTCCTCTATCGCAAACCCCCTTATCCTCATTGACGGCGCTTTTAATCCACTATGTGGCCCCCCCCGT
CCCTCGCTTGAAATCACCGGCTTTCATCCCCCTATCCCATTCCCCCACACCCTTCATGGTGCTGGGTCCCCCCGA
CCCCCTTTTCTCCACTCATAGGCCAGACACCTCATCTATCTACGAATAACCCCGCCGCCCCCTCCTCATTTTTAT
AATAA

Figure 37

NNNNNNNNCCCCCGCACTGGACCCCGCGGTGGCGGCGNNNNGNNANGNANTNTTGNTTTGTAANATGTTTGATAT
AGTTTGAAGGTATTATGTTANTTTAATTATGAAAAGTGATGTAATNTGGTAGTAATGTAGTATTTAAGATAAAGG
GGTTTAGATAGTAAAGATAGAAAAAGAATAAAAAAAATATTGATTGGATAATTAAAAAGAGATTTTGGTGATGGG
GATGAAGATCTATATAGAGTGTGATTGGTAGGGTTGTGGAGTATGAGTTGATGATTATGGGAATGATAAGGATGT
AGTTGGTGGAGTATTATTGAATTTGGTATTAAAAGTAATGGATAAAGGTGGGTGAAGGAATTTTATTGTACAAAA
ATGGTTGGGGCGTCTCACGGGGGTGAGTCAACTTACCCTATTAGACGTTTATACTCTCTATCACATCTGGGCGTC
ATGACAACAAATAGCCGCCGCTATTCCCAAGTCCCTCAACTGGGTGGCGTGATACGCGCCCGGGACCCCCCCTTT

AACAGTCGTTTGCCCGCGGGAGTGTTGTATGCTTCCCCCTTGACGCCAGTGCAAAAACCTAGATCCTAGGCCCCT
AACGCAATTATTATGACACTATCTCACACCATGGGCATGCGGGCATTCACGTCGATACCATTAACCTTGTTTATT
TCCCCTGTGTTGCGACCAATATTGTTTTTTAGGCCAGAGCCTTTACTCAAGGGGTTTAGCCATTTCCGCGCCCGT
AGCATACGCCATCCCCTCTCCTAATAGTAGCATTAACTGCAACGAAGACATCCTACACGTCCCTGTTATACATCA
TTCCACACAAATTTTCGTCCCCCAACTACCTATGATTTCCCCTAACATTACCTCAAACTATCGTCTCTACAACTG
AGGAGTAATACCACCCGTACAACGTCACAAGAATGGCTAATTTCTAAAACATGCGATAGCCTGCGATAGACTAGA
ATACACAATTCATCTACAAAAAAAATCTGACCCAATGAAATTAATAAACACAATAATGACAATACCACATTGCCC
TACACACCAGCTACAAAACCATTC

Figure 38

CGGCCGAGGTACATTCACTTAAATATAATAATGATGTTATTAATAAACGTTAGCTTGAGATCAAAGTGTCAACAA
GGCATTCTTTTCTCTTTTGGTGGAGGTAGATTTCACCCACATATTTTATGCATCTATAACAAGTCCCACATAGCT
GGCTGATTACTGAGCTTTGAGCAGGTAGCACTGGTCACTATGCACAGATTAGTATGATTACTGAGCTTTGAGCAG
CTTGAACAGATCACTATGATATAGGCTCTGGAAAGGTTGTGCAGATATGCCTGATGCCAAGGCACTGGCAAAATT
GTTGGTAGGCTGGCAAGTTAGTTCCTTCTCAAGGAAAAAAAAGTGTGAATGATCCTATAGGTTACTAGACAGACA
TGCAGTTTCCCTTTGGAGAAGGTTGACTTTGGACTTTGAGAAATACATTTTTTTTCTTTTAATCTAAAGAGATTT
GAAAATAATAGATGTCCAATTTTTGTTTACCTATTCTGAACTATAAGGCACATTGCTTTGTTCTTCTGTGCCTCA
GTTTACCCCTTTTAGTAAAGGAGGTCTATTTTAATCAGTGAGAGCTTCAGGTAGCTGGAGGAATGCTTCAGGAAA
CACAAAACACAATGAAAAAATTGTTGAATCTTAAGCTGCATGTTGTGGAAAAGAACTGTA

Figure 39

GCAGGTACGCTGTAACCTCATCTACTTCTGATGTTTTTAAAAAATGACTTTTAACAAGGAGAGGGAAAAGAAACC
CACTAAATTTTGCTTTGTTTCCTTGAAGAATGTGGCAACACTGTTTTGTGATTTTATTTGTGCAGGTCATGCACA
CAGTTTTGATAAAGGGCAGTAACAAGTATTGGGGCCTATTTTTTTTTTTTTCCACAAGGCATTCTCTAAAGCTATG
TGAAATTTTCTCTGCA

Figure 40

GAGGTACNNTGATGTCTGAGTAAGTAAAAAAAAAAATTGCTTACAGAAGTGACCAGATATTTATATATAAAACAA
CTGCTGTGTTTCATATCTGCACCTCAGATTTTTATACCTTTCACTTTAAATTCTACTAGCTGAGATAGACGACTA
TACAACAACTAAAGAAAAAGAAAAAATATTATAATATCTCATGTATCTAGTGTTACAGAGAAGTTCCCATCAACT
GCTGCATCAGTTAGTTAACTGGAGGAATAACACGAACTATGAATAGATGACCATTTTTTCAGAGAAGTAGAATAA
AATAAAATTCACCAAATACAATAAAATAAGTAATATTTTTAAAATAAGTAGCATAAAATATAATAAAATTCAGAT
AGAGAAAATGAACAGGGTTCAATTTTTTTTCTGATGTCAAGTTACCATTTGTTTCCTATTCATGGAGTTGAAGTG
ACTAAACTTTTTGATAATGCCAGATTTATTCATAATTCCTGGATAAAGGAATTTAATGAAAAAAATATAATGACT
AGTATATTTCTAAGGCCATAAAACTGTCCCAAAGAAATAAACTGAAAAAGTGAGAAAATTAGGTTCACCAATCTG
TATGTATATAAAAGAGTAAAAGAACAGGAACAACAAAAAACCCACCCTCAGAAACAGAGTA

Figure 41

CGCGGTGGCGGCCGCCGGGCAGGTNACGCGGGGGCCGCATAGGCAAGCACCGGAA

Figure 42

AACCTGACGACTCTTCTTCTTGTAATGGCTGCCCTTTTCCTACCTGAGGCCGTCTTAGAGAAAGGGGCCAGTCTC
CTCTAATGCTCAGATTTCCCATAGTTGGCTTTTGCTGTGTCTCCTGCCTCAGGCAGTGTCATTTCTGGGAGCAGG
TGGTTGTAGTCCAGGCCCCTCCCCAGCAGGGTCTGCCCAGGCTCCTTCGAGCCCCTTTCCCCGCCTCCTCTCAGC
CTGTCCGGATGACAGTGTTCGCCTCCTGTTTAGACTGTACACTCTTCAGGGGTAGGGGTGCCGTCAGTTCTTCAA
TCAGCTGGCACACACTTGTATAGTGAAATGTTTACATGTGGGAAAACTCCGCCTTAGACAAACTA

Figure 43

GGCCGAGGTACCTTGCATGCAGTGGACACTCAGTATTTGCTGAATTAAATTCCTTCCTATGGATCCCTTCTGATT
TTTTTTAAGTGCCTCTAATACACATATCATTCTAGGGCTCATGCCACTTTTAATGTCATTTTCTAAAGGAAAATC
TTATCTATGATATTTTCCCTTATAAGAGATAGTTGTTTTGAGTAGGGTTTTTTAAAAGATAAAGGTAGTAGGAAA
TTTTTTAAAGCCTAAATATCAAATTCCTTTCCCTTTGGAGTTGGGGGAAGGAATGAAGGGGGAGCAACTTGCTCT

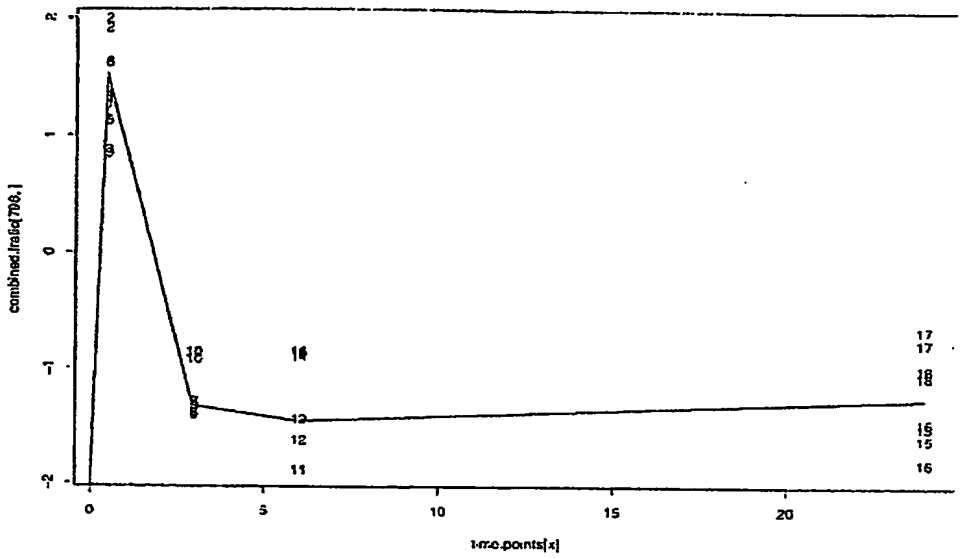
TTCATATGAGTTGGTCATAGCATGTAAGAACCAATCTTGAAATATCGTTTTTTTTTAATGGCTTATAATGTATTT
CTAGAAATACTTTGTA

Figure 44

AGGTACCGGCTGCGTGGCAGGTCTGGGTGTTGTGTGCGAGGACGTGGCCTTTGCACACCGCTGTGTTCTCAGAGG
TCCTTAGGAGATATTTTTTTTTTGTCTTAGGGGGACTGTGTTAAGTTCAGACAAATCATGCTGGGTGTGGAGAGAG
TCTGAAATACGTCAGTGAAGTAAGTAGCAGTGAGCGATTGTGAATGTGTAATGTAAATGGAAAACCGGGTTTTAC
CGTGTTAAGTTATTCACTAGGGAGCCAGTCGTAGTTCTTTGTAATCCTCTTTCTTCCAAACCTGCTTTGCTGAAA
GTTGCAGAAAAGGAAGTGTGTGGAGAGAAACAGAACCCTTCAGGGTGGGTCAGAGGACGCCATCCACAGTGGATT
CGTGTTCGTTTGCAGGTGGAAGCAGTGATTTTTAGGACCCACTGATTAAAAACAAACATTCCCAAGTGTCTCTGA
GAGATGCTGTTTATTTGTTAATTAAAAAGCTTTTTTTCTCTGTCTTTTAAATTATGGCTTTCATGTAATAAGGATA
TTTTTAGTGAAAAATTGTTTTCCTTTCAAATTACAGACCTTTTAAAAAAACTTAATTT

Figure 45

A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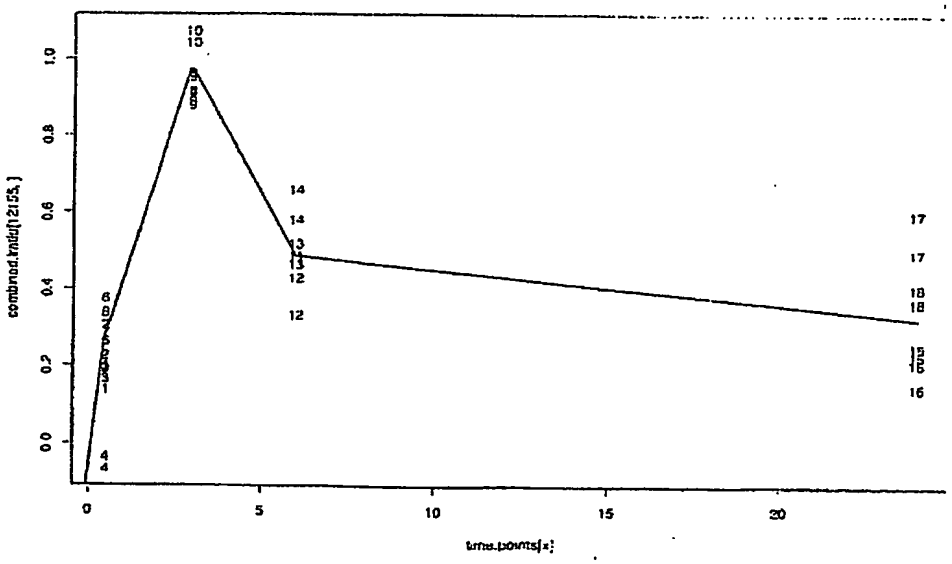
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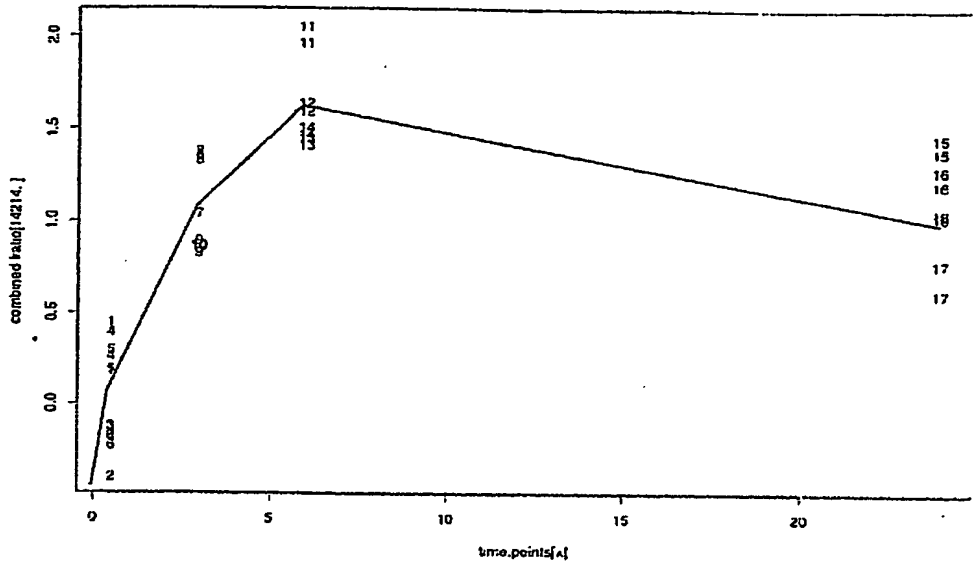
Figure 46

A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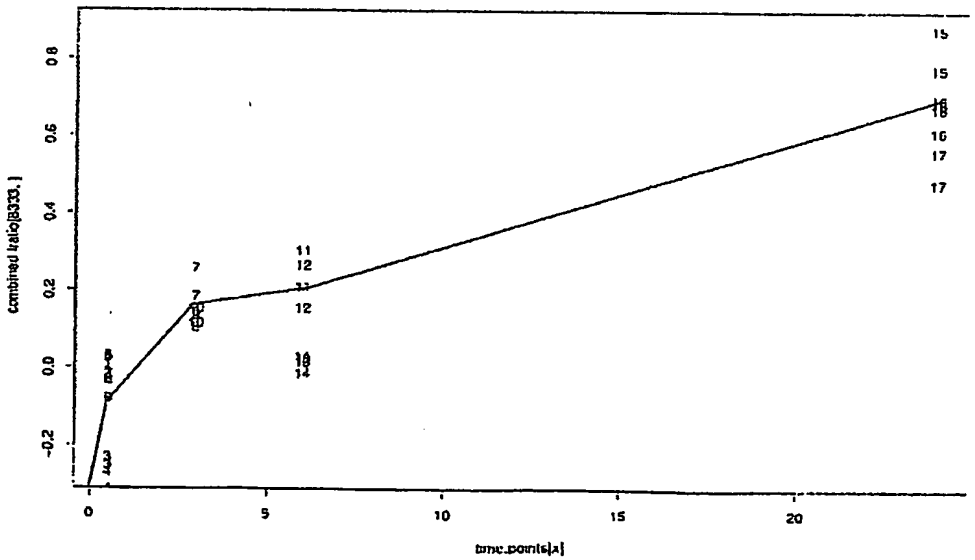
B


combined ratio[8333,]
time.points[A]